# INHERITANCE AND LINKAGE RELATIONSHIPS OF INDUCED MUTANTS OF COMMON BEAN, *Phaseolus vulgaris* L.

BY

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Dry seeds of common bean, *Phaseolus vulgaris* L., line 7 1404 were treated with 10 and 20 kilo roentgen (kR) of gamma-rays to induce plant mutations suitable for use as genetic markers in mapping studies. The mutations changed leaf shape and texture, and produced dwarfism and various chlorophyll deficiencies. Mutants with highly visible phenotypic characteristics were tested for inheritance. Two recessive mutants were selected for linkage testing in diallel crosses. Linkage was calculated using $F_2$ data, employing the product method. The maximum likelihood equation was employed to combine repulsion and coupling data to estimate a single linkage value. Two linkage groups involving 5 mutant characters were discovered. Round leaf (*rnd*), stipelless lanceolate leaf (*sl*), and dark green savoy (*dgs*) formed one linkage group (*dgs* — 21 — *sl* — 12 — *rnd*), while diamond leaf (*dia*) and progressive chlorosis (*pc*) formed the second linkage group (*dia* — 6 — *pc*). *Dgs* was linked (38 map units) to the

yellow wax locus, y, thereby [illegible] to the dgs rnd linkage gr[illegible] linkage group VII defined by Lamprecht. A coupling phase t[illegible] confirmed the linkage of rnd with dwarf seed (dy) at 23% recombination. Preliminary data suggest that the de character may be the same gene as Lamprecht's tensile (te), also of linkage group VII. If this is true, then the orientation of the dgs el rnd linkage group is determined with respect to y and F.

In one $M_2$ progeny 7 plants were discovered with a dwarf mutant phenotype. Tests of $M_3$ progenies, revealed outcrossing rates of 5 to 47%. Pollen staining tests of the mutant indicated an unusual pollen abortion rates. Seed set from plants grown in insect-proof enclosures were comparable to unprotected dwarf plants. Open flowers of dwarf plants manually crossed between 0630 HR and 1430 HR with either 'Sprite' or 7 1404 pollen resulted in cross-pollination of 2% to 86%. The frequency of cross-pollination declined with time of day. Evidence is presented supporting the hypothesis that delay of anther dehiscence is responsible for the elevated levels of cross-pollination. The proposed name for this mutant is dwarf outcrossing (dg).

# CHAPTER I
# INTRODUCTION

Extensive linkage maps that cover the chromosomes well have been produced for several crops, and for many other crops they are being developed. Knowledgeable use of linkage maps can be of considerable advantage in the design of breeding projects. Characters in close linkage relationships will especially benefit from such knowledge. For example, Rick and Fobes (1974) reported the close linkage of acid phosphatase isoenzyme to nematode resistance. Today many breeders make use of this knowledge to screen for nematode resistance. It is comparatively easy to select for the isoenzyme, while screening for nematode resistance directly is expensive, laborious, and subject to large experimental error.

Despite the potential value for linkage relationships, the development of linkage maps has been very slow for many crops. This is especially true for self-pollinated crops that produce minimal seeds per pollination. Many of the self-pollinating legume crops fit into this group. These plants possess flowers that are difficult to emasculate, and it is tedious to produce $F_1$ seed because seed yield per pollination are low. Thus, $F_2$ data is used in preference to the back cross data to study genetic linkage.

For common bean, the quality and quantity of characters available for linkage studies do not provide a particularly favorable experimental opportunity. Mendel (1866) reported on the inheritance

of testa and flower color and pod form. After [illegible] of Mendel's law, early plant geneticists, Shull (1908) and Emlen (1908a and b) used beans in many genetic studies. After this initial period, the use of beans in genetic studies declined. In 1961, Lamprecht published a comprehensive list of all known linkages. The list contained 26 genes distributed among 8 linkage groups. Since then, very few linkages have been reported. A major contributing factor to this history is the difficulty of working with the characters forming the present linkage groups. Many of the genes control color expression of the testa and corolla and are affected by complex complementary and modifier effects. This makes proper classification for genetic analysis very difficult. Although over 200 genes are described in the literature for common bean (Roberts, 1982), many of these genes are not convenient for the purpose of genetic mapping, and many others are not now extant or of questionable availability (difficult to obtain).

Artificial induction of mutations in plants with chemical or physical agents began in the 1920s (Stadler, 1928). The genetic variability produced was found to be similar to that produced naturally or spontaneously (Sigurbjornsson, 1983). The basic difference was the rate of occurrence, which is many times higher for induced mutations. Induced mutations are produced, selected, and grown with care, as opposed to nature, where many mutations are deleterious and provide very few or no progeny for a new generation. By selection of those mutations that could not survive in the wild, it

is possible to obtain new genetic markers. The principles are the same for most cultivated crops species which could not survive unaided by man. Since the natural genetic variability of common bean provides few characters suitable for linkage mapping, artificially induced mutations must be used to fill the void.

The identification of genetic variability that permits insects to cross pollinate beans would be valuable. Common bean is a highly self pollinated crop. Natural out crossing ranges from 0 to 10% (less than 2% in most areas) and is considered important only for seed purity since this level of out crossing is too low for producing hybrids. In the arid regions where bean seed is produced, the out crossing rates are extremely low (maximum of 0.013%, Tucker and Harding, 1975).

CHAPTER II
LITERATURE REVIEW

## Induced Mutations Produced by Various Mutagenic Agents in Common Bean, *Phaseolus vulgaris* L.

Numerous agents have been used to induce mutations in common bean (*Phaseolus vulgaris* L.). Among the most commonly used are gamma irradiation and ethyl methane sulfonate (EMS), a mutagenic derivative of mustard gas [Bis (2-chloroethyl) sulfide] (Sigurbjornsson, 1983). The intended use for the mutants and the methods of producing mutations varied among the researchers. Many investigated the spectrum of possible mutations, while others had specific types of mutants they desired (Moh and Alan, 1964, 1965, 1971; Moh, 1965, 1968, 1971, 1972; Stasova, 1971; Ruhalhaye, 1975).

Moh and Alan (1964) studied the effects of ionizing radiation on beans with specific emphasis on induced mutations. They postulated that since beans had a large reservoir of natural variation, it would be interesting to note the frequency and type of mutants that could be induced via mutations. A *dwarf* mutant was discussed by the authors, which was reported to be controlled by a single recessive gene. The mutant had smaller than normal primary leaves and extremely slow hypocotyl and epicotyl growth. Gibberellic acid treatment of the *dwarf* mutant at optimal levels induced internodal elongation and leaf expansion resulting in the *dwarf* plants being indistinguishable from the normal.

Moh and Allan (1965, 1971) and Moh (1968) reported 3 additional mutants: yellow mosaic, compact, and wrinkled leaf. Each was controlled by a single recessive gene. The yellow mosaic mutant was termed a chlorophyll mutation because of the 50% or greater yellow leaf surface (Moh, 1965). The mutant plant was completely sterile and thus the gene was maintained in the heterozygous state.

Frequency of occurrence for the wrinkled leaf mutant was high (Moh, 1968). This mutation occurred independently several times and all were morphologically similar. Mutant leaves were smaller and darker green and had a wrinkled appearance. Leaf cross sections revealed a higher density of chloroplasts in the palisade layer and generally thicker leaves.

Moh and Allan (1971) described what they termed a compact mutant. Mutant characteristics were: dark green leaves, petioles and rachis very short, and the peduncle extremely short. Seeds in matured pods were also highly compacted (expressed together).

Seed irradiation of the cultivar 'Blue Lake' with 1 to 10 kR of gamma rays, resulted in 4 morphologically discernible mutants in the $M_2$ generation (Mujeeb and Greig, 1973). Two semi-dwarf mutants were selected, designated SDM-1 and SDM-2. Both had determinate habit which contrasted with the indeterminate habit of 'Blue Lake'. The date of first bloom and maturity for both semi-dwarf mutants was delayed from that of 'Blue Lake'. LFHy, late flowering/high yielding was taller and had delayed flowering and maturity compared to 'Blue Lake'. The FSHy mutant (pod shape/high yielding) was characterized by flattened pods with twice as many seeds and increased

in total yield. All 4 mutants were stable over 4 generations. No significant difference in seed protein was observed for the mutant lines.

Irradiation has been used for the improvement of seed and protein yields. Direct increase in seed yield per unit area is the easiest method for increasing protein yield per unit area, and this can be done by increasing seed size or seed number per plant.

An alternative method would be to increase the protein content per unit of seed weight. Staneva (1971), using gamma irradiation to improve the protein content and amino acid composition of french beans, obtained higher yielding mutant lines. Hussein and Ibrouki (1975) increased the protein content and improved amino acid composition of haricot bean employing both gamma rays and EMS. Rubaihayo (1975) irradiated the haricot bean variety 'Mexico 142' and produced mutants with higher yield capacity with no increase in protein per unit weight of seeds.

EMS is the second major mutagen used on beans. Moh (1964) reported an EMS induced wrinkled leaf mutant which was morphologically identical to that produced by irradiation of bean seeds. Motto et al. (1975) treated dormant bean seeds with 24, 48, and 72 mM of EMS solution and reported the frequency of mutations and chimera formation in P. vulgaris. They found that 48 mM EMS induced the highest frequency of mutations (chlorophyll, morphological and seed-coat color). A glossy leaf mutant was described in a subsequent paper (Motto et al., 1975). It had a reduced leaf area and it was speculated that this mutant might tolerate high density planting and lead to increased seed

seed per unit area). Descriptions of this mutant are very similar to the wrinkled leaf mutant reported by Moh (1968). Both reported that mutant leaves were darker green, thicker than normal, and had a reduced lamina area.

Moh (1969, 1971, 1972) used EMS to induce seed coat color mutations of beans. He indicated the importance of such an undertaking by pointing out the superior disease resistance of many black seeded varieties and the reluctance of many people to eat black seeded beans in certain areas where beans are a major source of protein. The goal was to induce mutations from black to yellow, red, or white seed-coat and to use the correlation between green hypocotyl color and seedcoat colors other than black to screen for mutated seedcoat color in beans. Presumably, the superior disease resistance of the black seeded source cultivar would not be diminished by the seedcoat color mutation. He reported that some seed-coat color mutations had a monogenic recessive inheritance.

Hussain and Dasuki (1975) reported higher protein content and yield of a seed-coat color mutant produced from EMS treatment. When comparing mutation frequencies of EMS and gamma irradiation treatments, they found that only 1 out of 9 selected mutants came from EMS treatments. This result was contrary to most reports which usually found EMS with a higher efficiency rate. This ratio was attributed to the high degree of lethality produced by EMS in the particular bean variety used.

Mohan (1979) using EMS on seeds of 'Porrillo Sintetico' bean variety obtained a dwarf mutant. The dwarf plant grew relatively

slower than the normal plant, had dark green, wrinkled leaves, and shorter pods with seeds highly compacted. The mutant pods were shorter than the normal and the seeds within the pod were highly compacted. This mutant may have occurred at the same locus as the compact mutant of Meh and Alan (1971) because the descriptions are very similar.

Diethyl sulfate was used as a mutagen on beans (Davis and Frazier, 1964). Frazier and Davis (1965, 1966a) reported a dark green savoy mutant with a recessive inheritance pattern. The symbol dgs was tentatively assigned to the mutant. The mutant could be distinguished soon after germination by its very dark green and slightly savoyed leaves. The leaf margins were slightly curled and the leaf blade was much thicker in cross section. The second mutant reported was the silver mutant (Frazier and Davis, 1966b). The $F_2$ data revealed a 3 normal to 1 mutant ratio indicating control by a single recessive gene. The mutant was described as having silver colored leaves with severe plant stunting under high light intensity. The mutant had a normal growth habit under winter greenhouse conditions in western Oregon.

The genotypes of treated bean seeds differ greatly in their sensitivity to ionizing radiation and to various chemical mutagens (Osborne et al., 1963; Staneva, 1971; Al-Rubeai, 1981; Al-Rubeai and Godward, 1981). Different genotypes respond differently to the same mutagen in terms of injury to embryos in the $M_1$ seeds and the spectrum and frequency of mutants obtained in the $M_2$.

Seed size influences survival of the seed after radiation treatment (Staneva, 1971). He found that large seeded varieties were

more radiosensitive than were the small seeded genotypes. Al-Rubeai (1981) came to a similar conclusion working with wild and cultivated species of beans. He found a strong correlation between radiosensitivity and seed size and postulated that the primitive characters (e.g. small seeds and indeterminate habit) were associated with radioresistance.

Seed coat color also apparently influences the radiosensitivity of dormant bean seeds (Al Rubeai and Gudwerd, 1981). They concluded that lines with white seed coats were more sensitive than were seeds of pigmented lines based on the higher average treatment required to produce a 50% reduction in seedling height for the 51 varieties studied. Pigmented lines were on the average 15% more resistant than were white seeded lines. They found 'Koning Der Belgen', a black seeded variety, to be very resistant while 'Consteres', a white seeded variety, was relatively radiosensitive. Seeds of these varieties were treated with 5 kR and the effects in the $M_1$ generation were studied. Three weeks after sowing, the average $M_1$ seedling heights were 85.2 and 54.2% of the non-irradiated controls for 'KDB' and 'Consteres', respectively. When measurements of radiosensitivity were made as the percent survival in the $M_1$ compared to the respective non-irradiated line, a radiation exposure of 5 kR resulted in survival percentages of 106% for 'Koning Der Belgen' (KDB) and 66.7% for 'Consteres'. Crosses between the two parents produced $F_1$ hybrids as resistant as 'Koning Der Belgen', thus raising speculation that radiosensitivity was controlled by one or more dominant genes. The $F_2$ segregation data, however, revealed continuous variation for radiosensitivity. When

[illegible] $F_2$ seeds were separated by color, a clear difference in radiosensitivity was observed. Colored or pigmented seeds were resistant while white seeds were sensitive. Because of correlations between black seeds and radioresistance, the investigators suggested that the two characters were linked.

## Review of Linkage Determination for $F_2$ Segregation Data

Linkage relationships in P. vulgaris are usually calculated from $F_2$ segregation data because of the difficulty in making backcrosses and the limited number of seeds (5-7) produced from each cross.

Many methods of linkage determination for $F_2$ data have been formulated. The first was reported by Bateson and Punnett (1911). They relied on inspection to determine the closeness of fit between calculated and observed values. The phenomenon of linkage was thought to be an expression of symmetrical reduplication in the germ track.

Collins (1912, 1924) made use of Yule's coefficient of association for determination of linkage intensities. Yule's coefficient is a direct method of comparing the degree of relationship that exists between any two characters by coefficient of correlation. He stated that Yule's coefficient of association most nearly meets the requirements of a general method for linkage determination, because it can be computed directly from observed numbers and provide a probable error value. He incorporated Yule's coefficient of association into the theory of gametic coupling and assumed that correlations between two Mendelian characters were caused by attraction or repulsion between character units (genes). Bridges (1914) also proposed that

[illegible] coefficient of association was the most satisfactory method to use for linkage determination involving $F_2$ data. Adaptability to repulsions and coupling data and the calculation of probable error were reasons given for this statement.

Emerson (1916) presented a formula for calculating linkage intensities that was applicable to either repulsion or coupling data. Castle (1916) independently proposed an identical method. Later Fisher (1928) referred to both as the additive method. Gametic ratios were directly calculated from a dihybrid distribution and, by means of a formula, one could calculate the degree of linkage between both characters involved.

Castle (1916) simplified determination of linkage in the field with the development of a precalculated table of equivalent gametic and zygotic series. When linkage was suspected, inspection of the table would provide a quick estimate of the nearest gametic series.

Fisher and Balmukand (1928) pointed out a general limitation in the use of the additive model, viz. that only a portion of the available information was used. Thus, the efficiency of this method is quite low. Only for close linkage values does the efficiency of the additive model become quite high. Immer (1930) stated that the fraction of information utilized for various crossover percentages by the additive method may be obtained by dividing the variance of the maximum likelihood method by the variance of the additive method. He pointed out that at 10% crossing over in the coupling phase, the additive method is 96% efficient, while at 10% crossing over in the repulsion phase the efficiency is only 4%.

Woodworth (1923) expressed the need to determine linkage intensities from $F_2$ distributions where backcrosses were impractical to produce hybrid seed in sufficient numbers. He stated that Emerson's (1916) and Haldane's (1919) had developed formulas for calculating linkage intensities directly from monohybrid $F_2$ distributions or from dihybrid distributions, were not suitable for $F_2$ trihybrid distribution. The trihybrid distribution resulted from two factors, one of which was a duplicate gene system. Starting with Emerson's method, Woodworth developed a formula with which linkage values could be calculated where there exist duplicate or complementary factors.

Owen (1924) proposed the product moment coefficient of correlation, r, for calculation from $F_2$ data. He illustrated the relationship between r and crossing over intensity. Results obtained using this formula are more consistent and conservative. This method also has the added advantage of convenient algebraic manipulation. The crossing-over percentage or linkage intensity is estimated from r. The r and goodness of fit by chi-square test are determined in one operation. Single factors as well as duplicate genes, complementary factors, and simple backcrossing can be accommodated by this method.

Collins (1924) and Owen (1928) demonstrated that the product moment method is equal in efficiency with the maximum likelihood method, which is the accepted standard for judging efficiency of other formulas. The product moment method is the least affected by differential mortality of the zygote or gametes and has the smallest probable error for large sample sizes.

Immer (1930), searching for a method of linkage determination which was satisfactory for all ordinary linkage problems, reviewed several methods of linkage calculations. He concluded that the product method presented by Fisher (1928) and Fisher and Balmukand (1928) which was equivalent to the coefficient of association (Immer, 1930) was the best general method available when suitable tables were at hand. Fisher and Balmukand provided a table of crossover values with whole percentage points, 1 to 50, for Zeilel crosses involving 2 characters each controlled by a single gene. Immer (1930) calculated the values for coupling and repulsion linkage, resulting from crosses of characters that segregate in the following manner for a simple inheritance tests: 3:1 and 3:1, 9:7 and 3:1, 27:37 and 3:1, and 3:1 and 15:1. The probable error was also provided. He divided his crossover values into 0.005 increments for $F_2$ segregation data that resulted from the cross of 2 simply inherited characters.

Stevens (1933) pointed out the problems and disadvantage of inverse interpolations needed for the tables of Fisher and Balmukand (1928) and Immer (1930) where greater accuracy was needed, and he prepared a table sufficiently extensive for any experiment. He divided his crossover values into 0.001 increments for $F_2$ segregation ratios resulting from the cross of 2 simply inherited characters.

Linkage determined by the product method for $F_2$ coupling data is slightly inferior to the backcross method. $F_2$ repulsion data is found to be highly inferior to the backcross and coupling data, especially at close linkages. This is mainly due to the small number of double recombinants found in the repulsion phase when close linkages are

involved. The accuracy and reliability of linkage intensity estimates based on repulsion data approaches that of the coupling phase data only at 50% recombination.

The major limitation cited for the product method is that there must be 4 classes of data to obtain a linkage value. This is especially a problem with repulsion phase tests where a moderate population size (e.g., 1000 plants) is used to estimate a very tight linkage, which usually results in a missing class of data.

Haldane (1919) presented a formula for calculating the cross-over percentage from $F_2$ data and that for calculating the probable error. Fisher (1928) stated that Haldane could arrive at his formula only by using what he called the maximum likelihood method. Fisher (1928) and Fisher and Balmukand (1928) stated that the maximum likelihood method is all cases of linkage had the smallest possible error in theory for large sample sizes (greater than 1000).

The maximum likelihood method is general in its application and may be applied to any linkage data. This method may also be used to combine coupling and repulsion data sets to provide a single estimation of linkage. Homogeneity among the data sets can also be estimated with appropriate calculations.

Allard (1956) simplified the use of the maximum likelihood method by supplying scores for the maximization of the logarithm of probabilities, which leads to linkage estimation.

Recently several computer programs have become available for the determination of linkage recombinational parameters. Hanna et al (1978), Suiter et al. (1982) and Eppig (1983) employ the maximum

likelihood method which provides the best linkage estimates for $F_2$ segregation data. Hanna et al. (1978) have written their program with FORTRAN for a Control Data System Cyber 70 computer. They state that with minor modifications, the program will run on the IBM 360/370 computer. The program can handle class sizes up to four digits and a pooled total up to 9999. Suiter et al. (1983) used PASCAL to write their program, which is available for use with 3081 IBM mainframes through cards or magnetic tapes where a mainframe computer is available. The program is also available on floppy disc for use on IBM PC or Apple II personal computers. Epping (1983) has used FORTRAN IV to write his program designed for use on video terminals, and he states the program is "user friendly". Copies of all programs are available from the respective authors.

## Reported Linkage Relationships of *Phaseolus vulgaris* L.

Lamprecht (1961) summarized the known linkage relationships of *Phaseolus vulgaris*. He reported 26 genes in 8 linkage groups.

### Linkage Group I

Lamprecht (1947) observed the linkage between R and C both of which are color genes for the testa. R confers a light vinaceous color of the testa and is incompletely dominant (Tymbes, 1931). C is incompletely dominant and has varying effects depending on other genes present. C causes strong marbling of the testa in the heterozygous state. Linkage value between R and C is p = 0 units. The linkage was observed between R and Uc with p = 21.7 units (Lamprecht, 1948). Uc

with appropriate genes darkens the banner petal (Lamprecht, 1948). The Ins - Cor linkage relationship was defined by Lamprecht (1952) with p = 27.6 units; Ins with appropriate factors gives light buff or raw silk tints, while Cor darkens the corona around the hilum. Lamprecht (1961) demonstrated the linkage of Con - Cor - R thereby combining independent linkage groups R - C, R - Uc, and Inc - Cor into a single linkage group. The probable order is Con - Ins - Cor - R - C - Uc. The relationship of R, C and Uc is still unclear, and the possibility exists that Uc could be situated to the left of R.

Frekken (1936) reported a linkage relationship between C-c and M-m, which were factors for seed mottling. He stated that C-c and M-m were absolutely linked because no recombination occurred between the C and M loci. Later Feenstra (Yarnell, 1965) reported that C and M was the same as C reported by Lamprecht in 1932 and 1940; hence, no linkage relationship existed due to the single factor involved.

Linkage Group II

The first linkage group reported by Lamprecht (1932) was for the characters Fb - Ea - Da. These pod characters were: Fb - supplementary gene for pod membrane; Ea, ea - alleles for round and flat pods, respectively; Da - allele for straight pods. Linkage distances reported were Fb - Ea, p = 13, Ea - Da, p = 15; and Fb - Da, p = 22. Schreiber (1932) reported the distance between Ea and Da at p = 13%.

Linkage Group III

Brakken (1936) reported a linkage between B, which produces a grey-greenish brown seed coat without a hilum ring, with St for stringy pods. The crossover value for $F_2$ material was 23.9 units, while the backcross resulted in 27.9 units. Lamprecht (1961) and Yarnell (1965) reported the crossover value at 25 units.

Linkage Group IV

Smith (1947) while studying the inheritance of seedcoat color of 'Pinto' bean observed a linkage of 38% recombination between two color genes Rk and Br. Rk when combined with Sh, resulted in chassis or cream color tests; and when Rk was combined with P, Gri and J pinkish buff seedcoats resulted. The recessive allele rk with Sh gives tentaceous tests, the buff color of the 'Red Kidney' bean. Br interacts with P and Rk for brown seedcoats, while br with P and Rk gives green seedcoats and with P and rk gives pink seedcoats. Lamprecht (1961) considered Br to be identical to Och.

Linkage Group V

Linkage recombination of 31% was estimated between the characters sepal red and indeterminate axis. No was originally observed from the cross of Phaseolus vulgaris and Phaseolus coccineus (Lamprecht, 1948). The flower color was described as lighter than Salmon Red, with a

[illegible] linage. Ne produces a much darker reddish color of the flower buds. Fin determines indeterminant growth, while fin determines determinant growth of the axis.

Linkage Group VI

The Arg - Sal linkage group was reported by Lamprecht (1948) with p = 26.9 map units. Arg with Y produces "silver" or greenish gray pods, a characteristic which was formally designated s by Currance (1931). The recessive arg with y gives white pods. The Sal character with basic color genes P and Gri produces a salmon red flower color.

Linkage Group VII

The Y - Te - Mrv linkage group was established by Lamprecht (1952). The gene Y determined yellow pods, and te shortened pods to 5-8 cm and produced a narrowing of pod width (Lamprecht, 1952). Mrv flattened the ends of seeds, and shortened the distance between funicles. Recombinational distances were Y - Te, p = 32.2 units; Te - Mrv, p = 13.4 units; and Y - Mrv, p = 42.6 units. Lamprecht (1955) added Sur, Cav, and P to the Y - Te - Mrv linkage group. Sur is a dominant gene that causes leaves to point downwards (Lamprecht, 1937). This had the same effect as Xan (Lamprecht, 1961). Cav causes a wrinkling of the testa radiating from the caruncule. Expression in the heterozygote is less distinct, which indicates incomplete dominance. P is the basic color gene along with Gri. P without Gri and color genes is colorless as is p. P may be identical or equivalent to the A of Tschermak, Kesimon and Sirks (Feenstra, 1960).

The map distances are Sur - Y, p = 28; Y Cav, p = 31; Cav Te, p = 8; Te - Mlv, p = 13; Mlv - F, p = 18 units.

Linkage Group VIII

Lamprecht (1948) reported the V - Unc - Ia linkage group. Linkage values were V - Unc, p = 35.4 and Unc - Ia, p = 12.5, V with F/Gri produces a pale glaucescens testa (bluish-green or yellowish-green color) without a hilum ring. According to Feenstra (1960), V is the equivalent of B (Shull, 1908, Tschermak, 1931), F (Kooiman, 1931), G (Shaw and Norton, 1918), and Z (Sirks, 1922). Unc in the presence of other genes, viz. Aeq, Uc, F, T, E, and Rst, darkens the banner petal of the flowers to varying degrees, depending on which dominant genes are present. Ia gives "parchment" versus Im for "tender pod"

Linkage relationships between systemic chlorosis and water soaked leaf reaction to inoculations with Pseudomonas phaseolicola was established by Coyne et al. (1971). Values of 15.0 and 13.5 were obtained from the coupling cross of 'Gallatin 50' x 'G. N. Nebraska' and 'Gallatin 50' x P. I. 150414, respectively. For the first cross, water soaked reaction and systemic chlorosis reaction were both controlled primarily by a single dominant gene. For the second cross both reactions were each controlled by a single recessive gene.

Linkage between genes controlling tolerance to Xanthomonas phaseoli, common bean blight, and genes controlling delayed flowering were suggested by Coyne et al. (1973) from the cross 'G. N. 1140' x 'G. N. Nab. 1 sel. 27'. No mention of a linkage value was made, nor was the number of plants found in each class given.

Valladares-Sanchez et al. (1979) reported linkage between blight tolerance and the genes determining late maturity and indeterminate plant habit in the cross 'G. N. Neb. 1 sel. 27' x 'Guali'. No linkage estimate was given, but from the $F_2$ segregation data provided, a linkage estimate of 26% could be calculated. The results of these experiments are confounded by the occurrence of early maturing plants from parents that were late to moderately late in maturity.

Coyne and Schuster (1974) observed a linkage of 8.4% between the characters for determinate plant habit and early maturity from $F_2$ segregation data resulting from a coupling cross, 'Gallatin 50' x P. I. 207262. Determinate plant habit was recessive to indeterminate, while early maturity was controlled by a single recessive gene. In contrast, Valladares-Sanchez et al. (1979) reported a linkage among the genes controlling late maturity and indeterminate plant habit from the cross 'G. N. Neb. 1 sel. 27' x 'Guali', but did not provide a linkage estimate. A single dominant allele produced the indeterminate habit and homozygous recessive alleles produced the determinate habit. $F_2$ segregation data revealed that late maturity was controlled by 2 dominant genes, while early maturity was reported to be controlled by duplicate recessive genes.

Linkage recombinational value of less than 5% was estimated for the indeterminate habit with at least 1 of the 2 genes controlling days to flowering in French bean (Freire Filho, 1982). He reported a gene action of incomplete dominance for late flowering.

Resistance to Sclerotinia sclerotiorum was observed to be linked to late maturity (Coyne et al., 1977). Lateness was controlled by 2

complementary dominant genes, one of which is linked to [illegible] No recombinational value was given.

York et al. (1977) reported an association between dark seed-coat color and resistance to Pythium ultimum. They did not indicate the degree of linkage, mentioning only that it was possible to select white seeded bean lines with Pythium resistance.

Linkage between flower color and variegated color pattern of foliage was discussed by Coyne and Steadman (1977). Flower color, violet or white, was determined by two genes with complementary gene action. The variegated foliage pattern was controlled by duplicate recessive genes. No linkage estimate was given.

Phaseolin polypeptides are linked to group B proteins, and G2/albumin polypeptides were linked to the group F proteins (Brown et al., 1981). The phaseolin proteins constitute about 40% of the total seed protein, and they are low in sulfur amino acids and have poor digestibility. The globulin-2 (G2/albumin) protein constitutes between 5 to 12% of the total seed proteins and contains hemagglutinating lectins which have antinutritional effects. The proteins, A, B, D, E, and F, are extractable proteins characterized by their identifying isoelectric points and molecular weights. Nutritional and digestibility properties of these proteins are unknown. Crosses of 'Sanilac' x 'Contender' and 'Tendergreen' x 'Sanilac' revealed linkage values of 32% and 34%, respectively, for the association of Phaseolin with group B polypeptides. 'Sanilac' x 'Contender' and 'Tendergreen' x 'Sanilac' revealed linkage recombination of 5 and 9%, respectively, for the association of G2/albumin with group F polypeptides.

Provvidenti et al. (1983) identified the tight linkage between genes for resistance to blackeye cowpea mosaic virus (BICMV) and to cowpea aphidborne mosaic virus (CAbMV). Since the alleles for resistance were always found together, very close linkage was suspected. They have not ruled out a single factor for resistance to both BICMV and CAbMBV in bean line PT1. They chose to consider the two factors for resistance as independent entities until analysis of the nucleotide sequence homologies of the viruses and the resistance genes proves otherwise. They feel that the theory for 2 factors will be correct because the two viruses are very different in antigenic response.

## Estimation of Natural Outcrossing Reported For *Phaseolus vulgaris* L.

*Phaseolus vulgaris* L. is a highly self pollinated species due to chasmogamy (Weinstein, 1926), proximity of anthers and stigma during anther dehiscence, and simultaneous anther dehiscence and stigma receptivity (Webster et al., 1977). Weinstein (1926) reported that the flowers of common beans opened between 1:30 and 2:30 a.m. under greenhouse conditions. Pollen grains are shed at this time with fertilization taking place in about 8 or 9 hours after pollination. Weinstein reported that the rapidity of the fertilization process following pollination may be dependent to a considerable extent on high greenhouse temperatures. He cites Martin (1904) who observed similar characteristics in *Trifolium pratense*.

Webster et al. (1977) studied the reproductive structures of common bean and observed anthers directly appressed to the

stigmatic surface of flowers in the white bud stage. The white bud stage occurs just prior to floral opening, during which time anthers dehisce and release mature pollen grains. The coincidence of anther dehiscence and stigma receptivity was found to contribute to self pollination.

Darwin (1857) noted that when bean plants were netted to exclude bees, they produced a number of pods equal to unnetted plants. The same observation was made by Free (1966, 1970) nearly a century later. Free caged all plants and found cages without bees produced as many pods and seeds as cages with bees. No differences in seed size or weight were found among cages.

Although beans are reported to be highly self-pollinating, outcrossing or natural hybridization has been reported to be as high as 10% or more. In Nebraska, Emerson (1916a) observed the rate of natural hybridization varied from 0 - 10% in his field plantings. In contrast, Kristofferson (1921) deliberately tested for the percentage of outcrossing by planting diffasent varieties in adjacent rows. He observed on average of 1.05% outcrossing, with shell beans showing a high outcrossing rate of 17.7%. He noted varietal differences in the percentage of outcrossing. Kristofferson observed an average outcrossing rate of 0.8% for snap beans and 1.42% in field beans. He did not discuss any reasons for the apparent difference.

Over 15% outcrossing was reported by Mackie and Smith (1935) for 'California Pink' bean grown at King City, California, whose field grown seeds were tested. They indicated that the outcrossing could have occurred over several generations since the farmer kept his own

seeds to plant the following year. In a test conducted at Berkeley, California, in which 60,000 plants were grown, only 8 outcross plants were observed (0.013%). In another test, 0.73% outcrossing was observed in pure white seeded varieties grown in rows adjacent to colored varieties. They concluded that outcrossing usually occurred between plants in adjacent rows. However, no attempt was made to determine the outcrossing percentage between plants within the row.

Outcrossing of 2.70% was observed between 2 dwarf bush bean varieties, and 5.11% outcrossing occurred between 2 pole bean varieties when grown in adjacent rows (Barrons, 1938). Barrons determined that increased distances between the rows decreased the percentage of outcrossing. For distances of 1, 2, 3, 5, and 9 yards between rows, Barrons (1939) reported outcrossing rates of 8.26, 7.52, 4.90, 4.78, and 2.63%, respectively, for Alabama No. 1 and Alabama No 18 bean lines grown in adjacent rows. He reported these values to be higher than those of most reports, and suggested that in the southeastern United States, the degree of natural outcrossing is higher due to environmental and pollinator factors.

At closer spacing distances, Alan and Mok (1968) observed that space plantings of Colombia-102-N and 5681 grown at 25, 50, and 100 cm between rows, resulted in outcrossing rates of 0.05, 0.20, and 0.18%. A reduction of total plant area of the seed parent available for insect visitation was suggested as the reason for the low outcrossing percentage at 25 cm spacing. However, tests with similar between row spacings were reported by Atunes et al. (1973). They reported outcrossing rates of 10.6 to 6.2% for 20, 50, and 80 cm spacing at

Pelotas, Brazil. Outcrossing rates of 8.7 to 8.3% occurred for the second year of plantings at the same spacing distances.

Varietal differences are known to influence the potential degree of outcrossing in beans. Barrons (1938) concluded that the degree of outcrossing of some varieties is inherently higher than others, as in the case of Alabama No. 1 and Alabama No. 18. Tucker and Harding (1975) reported estimates for outcrossing ranging from a minimum of 0 to a maximum of 0.013% for 12 common bean cultivars grown at Davis, California. It was concluded that the environmental conditions favored the selfing of beans over outcrossing.

The frequency of natural hybridization between P. vulgaris and P. coccineus was observed to range from 0 to 6.7% (Rutger and Beckmann, 1970). The level of outcrossing was dependent upon parental combination, for which P. vulgaris usually was the seed parent. The cultivar 'Tendergreen' had the highest frequency of outcrossing with P. coccineus lines; the average was 3.7%. Close examination of floral parts from 'Tendergreen' flowers revealed 49 atypical to 288 normal flowers. Atypical flowers were incompletely developed with keel and/or petals deformed, thereby leaving the reproductive parts exposed to insect pollinators.

CHAPTER III
INHERITANCE AND CHARACTERIZATION OF GAMMA RAY
INDUCED MUTATIONS IN COMMON BEAN, Phaseolus vulgaris L.

## Introduction

A total of 211 genes have been characterized in common bean Phaseolus vulgaris (Roberts, 1982). Most of these genes have not been assigned to linkage groups. Lamprecht (1961) reported 23 genes in 8 linkage groups. Few linkages have been reported since then. A major obstacle that prevents the exploitation of existing linkage groups is the difficulty in working with these characters. Lamprecht's map included 11 seed traits, 7 pod traits, and 3 flower traits. Many of these genes control color expression in the testa and corolla, and are affected by complex complementary and modifier effects. Proper classification, needed for genetic analysis, becomes very difficult when these genes are involved in linkage studies. Another difficulty with using color and color patterns is the subjective nature of these types of characters (Yarnell, 1965). Color can also be influenced by environmental conditions and genotypic background.

Many of the 211 reported genes have poor phenotypic quality for the purpose of genetic mapping. This may contribute to the lack of genes mapped in common bean. With additional gene markers, identification of bean chromosomes through linkage mapping will be possible.

Chemical compounds and ionizing radiation have been used to induce mutations in many crops, notably tomato (Rick, 1974), pea (Blixt, 1974), and rice (Sakai and Suzuki, 1954).

It is the intent of this experiment to induce mutations by gamma irradiation and to select those that would make gene markers suitable for genetic linkage mapping.

## Materials and Methods

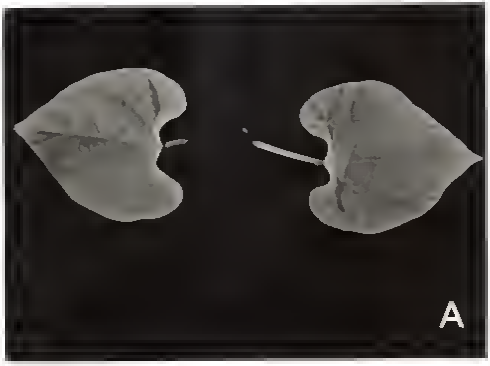
Plant material employed as the line of origin for this study was Florida bean breeding line, 7-1404 (Figure 3-1A and 3-1B). This is a nearly homozygous, black seeded, dry bean line with half runner growth habit. Line 7-1404 was chosen for the superior root rot tolerance needed for Florida conditions.

Dry seeds were treated with gamma irradiation from a Gammator Model M Cs137 source located at the USDA Insects Affecting Man and Animals Lab, Gainesville, Florida. Treatment levels consisted of 10 or 20 kilo roentgen (kR) exposure at a dose rate of 1952 R per minute. Seeds were held in paper sacks during irradiation.

Treated seeds were planted in field plots and allowed to produce $M_2$ seeds. Dry seeds were harvested from individual $M_1$ plants and stored in individual seed envelopes. For the 10 and 20 kR treatments, 255 and 157 individual lines, respectively, were harvested.

A single set of 100 $M_2$ seeds from each individual $M_1$ plant was planted in the field in 1980 at the University of Florida, IFAS Horticultural Unit in Gainesville. The plants were grown on raised beds spaced 1.2 m apart. There were two rows per bed spaced 30 cm apart, with plants 10 cm apart in the row.

Figure 3-1. Florida breeding line, 7-1404. A. Seedling of 7-1404 about 10 days from sowing B. Trifoliolate leaf from 7-1404 at first anthesis.


A


B

When the primary leaves were fully expanded, screening for mutants began. Screening continued on a weekly basis until plant maturity. All mutants were recorded. Characteristics suitable for genetic markers were labelled for later identification when $M_3$ seeds were harvested.

Characters suitable for genetic markers possessed all or most of the following characteristics:

1) Early expression of the mutant character; i.e., a seedling marker, one that is expressed in the primary leaves, cotyledons or hypocotyl.

2) Distinctive phenotype, making it easy to classify segregating progeny.

3) Good vegetative vigor and adequate reproductive capacity to maintain the mutant line.

4) Simple mendelian inheritance usually controlled by one recessive gene.

Nine $M_3$ plants of each selected mutant were grown in a greenhouse and evaluated for the first 3 criteria. Those selected were crossed with 7-1404 pollen to study the inheritance of the mutant characteristic.

Six hundred $F_2$ seeds were planted in the field to test for inheritance. Planting was done as previously described for $M_2$ seeds. The observed $F_2$ ratios were tested by the chi-square goodness of fit test to either a 3:1 or 15:1, normal:mutant ratio. Probability values for (P) were determined using a programmable calculator.

Since no formal rules for gene nomenclature and symbols exist for *Phaseolus vulgaris*, the rules for *Vigna* were employed. Fery (1980) proposed that the set of gene nomenclature rules for Cucurbitaceae devised by Robinson et al (1976) be adopted for *Vigna*. Robinson et al. based their system on that of tomato (Barton et al., 1955; Clayberg et al., 1970) and on the recommendations of the International Committee on Genetic Symbols and Nomenclature (Tanaka, 1957).

Leaf area determinations were made on 5 field grown plants of each morphological mutant at first anthesis. Measurements were made on each trifoliolate leaf with a Li-Cor leaf area meter, Model LI-305A. Each leaflet was removed from the rachis or petiole and summed for the total leaf area.

## Results and Discussion

A number of mutants were selected from the $M_2$ population that possessed visible phenotypic characteristics that possibly made them useful as genetic markers. Further tests for character expression, fertility and mode of inheritance reduced this list to 10 mutants which are discussed in detail (Table 3-1). Many mutants were rejected for low fertility. A few advanced selections turned out to be more difficult to classify in segregating materials than anticipated, and a few had abnormal inheritance patterns. The inheritance data for all unselected mutants are listed in Appendix A.

All inheritance tests were done using 7-1404 as the pollen or male parent. This made it possible to distinguish dominant gene characters from recessive characters in the $F_1$ and to identify

Table 3.1 Proposed names and gene symbols for marker mutants induced by gamma irradiation of dry bean seeds.

| Name | Gene symbol | Treatment level (kR) |
|---|---|---|
| Round leaf | rnd | 10 |
| Dark green savoy leaf | dgs | 10 |
| Diamond leaf | dia | 30 |
| Chlorotic cup leaf | cc | 20 |
| Dwarf outcrossing | do | 10 |
| Stipelless lanceolate leaf | sl | 20 |
| Chlorotic stem | cs | 10 |
| Progressive chlorosis | pc | 20 |
| Silver leaf | sil | 10 |
| Yellow green | yg1, yg2 | 20 |

cytoplasmic or maternal characters in the $F_2$. The inheritance tests for the selected mutants revealed that all were controlled by a single recessive gene except for yellow green, which appears to be controlled by duplicate recessive genes (15:1 ratio). The silver leaf mutant is probably controlled by a single recessive gene that has reduced transmission rates due to an environmentally sensitive gametophytic effect (Table 3-2).

Dark Green Savoy (dgs)

This mutant is a seedling marker, which is readily classified when the primary leaves are fully expanded (Figure 3-2A). Leaves of this mutant are dark green with a savoy texture and glossy epidermis. Trifoliolate leaves of dgs are smaller than those of 7-1404, with an average leaf size of 51 $cm^2$ as compared to 94 $cm^2$ for 7-1404 (Table 3-3). The leaves of dgs are more responsive to light than are leaves of 7-1404, orienting themselves to receive maximus light on the leaf lamina at lower light levels.

Frazier and Davis (1965a), Meh (1968), and Motte et al. (1979) have reported mutants induced by radiation or chemicals that are similar to our dark green savoy. Frazier and Davis (1965, 1966) induced a mutant termed dark green savoy using diethyl sulfate, which was controlled by a single recessive gene. The mutant type was easily distinguishable soon after emergence. Because of very similar descriptions of this mutant to our mutant, and the precedent it takes, we applied the same dark green savoy to our mutant. Dr. J. R. Baggett (personal communication, 1983) indicated seeds of the dgs mutant no

Table 3-2. The $F_2$ segregation ratios and chi-square tests from crosses between 7-1404 and 9 induced bean mutants.

| Mutant | $F_2$ segregation Normal : Mutant | $\chi^2$ (3:1) | P |
|---|---|---|---|
| Round leaf | 422 : 139 | 0.0148 | 0.90317 |
| Dark green savoy leaf | 415 : 141 | 0.3259 | 0.56809 |
| Diamond leaf | 444 : 141 | 0.2513 | 0.61616 |
| Chlorotic cup leaf | 290 : 85 | 1.0889 | 0.29671 |
| Dwarf outcrossing | 176 : 59 | 0.0014 | 0.97015 |
| Stipellate lanceolate leaf | 151 : 46 | 0.2860 | 0.59279 |
| Chlorotic stem | 437 : 148 | 0.0278 | 0.86758 |
| Progressive chlorosis | 427 : 141 | 0.0093 | 0.92317 |
| Silver leaf | 319 : 78 | 4.8830 | 0.02710 |
| Yellow green | 535 : 37 | 0.0466* | 0.82905 |

*Chi-square test ratio of 15 normals: 1 mutant used.

Figure 3-2. Dark green savoy leaf mutant (dgs)
A. Seedling of dgs about 10 days from sowing, note the shiny or glossy leaf epidermis
B. Trifoliolate leaf from dgs at first anthesis showing the savoying and glossy leaf epidermis.


A


B

Table 3-3. Variation of leaf size, leaf number, and total leaf area for T-1404 and 5 mutant bean lines

| Mutant name | Means and SE of means | | |
|---|---|---|---|
| | Leaf size ($cm^2$) | Leaves/plant (number) | Leaf area/plant ($cm^2$) |
| T 1404 | 93 94 ± 15 5 | 24 25 ± 1.64 | 2278 14 ± 373 7 |
| Round leaf | 91 10 ± 15 4 | 29.67 ± 4.40 | 2702.86 ± 464.2 |
| Stipelless lanceolate leaf | 51.91 ± 8.0 | 44.17 ± 7.60 | 2293.20 ± 398.1 |
| Dark green savoy | 51 59 ± 13.4 | 32.93 ± 9.00 | 1693.78 ± 441.6 |
| Diamond leaf | 35 70 ± 9.4 | 41.67 ± 10.1 | 1487.50 ± 392.1 |
| Dwarf outcrossing | 34.44 ± 6.3 | 140.17 ± 27.0 | 4827.40 ± 881.6 |

longer exist in the seed collection of Corvallis, OR and probably does not exist elsewhere. This makes it impossible to test for alleliem.

Nah (1968), using continuous levels of gamma irradiation in an exposed gamma field, obtained a wrinkled leaf mutant from Mexico '3OR'. The $M_1$ parent was exposed to 75r of gamma rays daily for 80 days. The wrinkled leaf mutant was controlled by a single recessive gene. Expression in the primary and trifoliolate leaves which he reported to have a wrinkled appearance some after emergence. Leaves were darker green and generally smaller than normal. Histological observation of cross sections of mutant leaves showed them to be about 24% thicker than normal. Cells of the palisade layer were more densely arranged than in the normal. Since these cells are filled with chloroplasts, they inferred that the dense arrangement gave rise to the dark green color. Seeds of this mutation are not known to be in any collection and are presumed to be lost.

Motto et al. (1975) reported an EMS induced mutation, glossy (gl), and indicated control by a single recessive gene. The mutant was found in the $M_2$ population after the cultivar Royal Red had been treated with EMS. All indications from descriptions and photographs point to the probability that dark green savoy, wrinkled leaf, and glossy are the same mutation as our dgs.

It can be speculated that this mutant phenotype might have potential not only as a genetic marker (Nah, 1968) for linkage studies, but also for improving yield potential. Motto et al. (1978) described the growth analysis of glossy as a reduced leaf area mutant of common bean. They found that the net assimilation rate was 62.3%

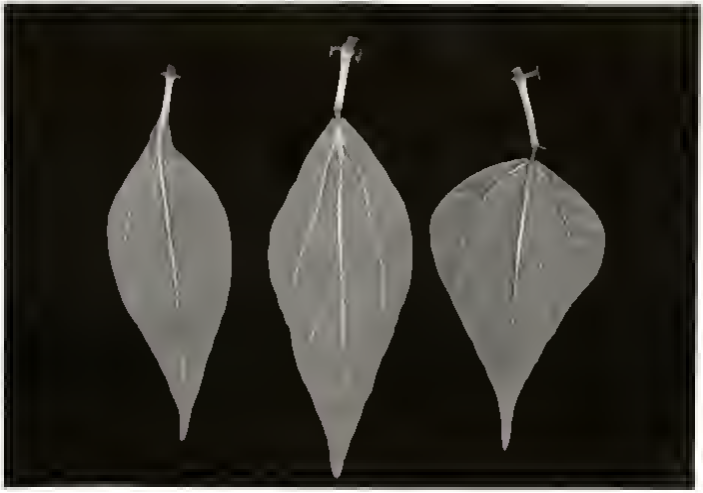
greater than normal under similar degrees of illumination. They concluded that, per unit area of leaf surface, glossy was were efficient than normal leaves in assimilating photosynthates. They speculated that the distribution of leaves along with reduced size within the canopy favors penetration of light to the lower levels of vegetation providing better photosynthetic activity over all leaves. Although the yields for glossy are lower than for normal plants at equal plant spacing, it was suggested that compensation could be made by growing more glossy plants per unit area. Provided that the net assimilation rate remained the same under these conditions, increased yield is potentially possible if glossy is utilized in breeding programs.

Stipelless Lanceolate Leaf (el)

This mutation produces a change in the normal leaf shape to a lanceolate form with loss of stipels from the rachis of the terminal leaflet (Figure 3-3). Trifoliolate leaves produced at nodes 1 through 5 ± 2 had reduced but distinct pulvinar regions with the termination of the leaflet lamina at this point (Figure 3-3). The terminal leaflets of nodes 5 ± 2 and above had highly reduced pulvinus regions with the formation of wings down the rachis (Figure 3-3).

Leaflets of el lacked phototrophic movement to align the leaflet blades for maximum light interception. The proximal end of the leaf blade is normally held parallel to the ground which causes the distal end to droop downward, possibly resulting in lower photosynthetic activity. Terminal leaflets were observed hanging on to the petioles

Figure 3-3. Stipellass lanceolate leaf mutant (sl). On the left is the terminal leaflet of 7-1404 with stipels. In the middle is the terminal leaflet of sl observed at nodes 1-5 ± 2 (lateral leaflets removed). Note the distinct pulvinus region similar to that of 7-1404 (P) On the right is the terminal leaflet of the sl mutant observed at nodes 5 ± 2 and above (lateral leaflets removed). Note the wing formation down the rachis and the absence of a distinct pulvinus region.



after leaf senescence. The reduction of the pulvinus region may play some role in the metabolic sink due to the importance of the pulvinus region to leaf abscission (Brown and Addicott, 1950)

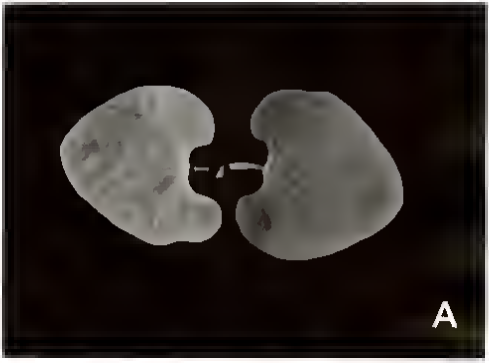
This mutant, when grown under greenhouse conditions, tends to express a vining habit. Plants up to 2m in length were noted when trellis supports were provided.

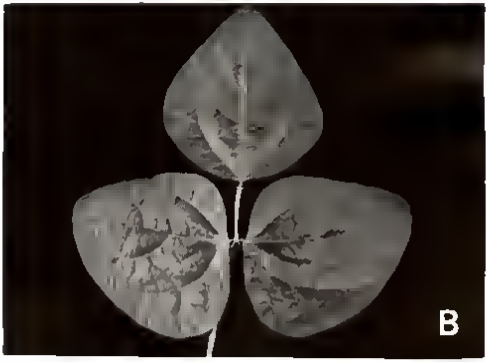
Blue flowers and a possible translocation were found with the sl mutant in the $M_2$ generation. These blue flowers were found to be independent of sl and inherited by a single recessive gene. Translocation is suspected because of semisterility of pollen and seeds which would result from translocation heterozygotes (Schulz-Schaffer, 1980). Semisterility was observed in some $F_1$ plants of sl crosses, supplying the evidence of a translocation.

Round Leaf

This mutant produces a rounded apex of the primary leaves and lateral leaflets of the trifoliolate leaves (Figure 3-4A and 3-4B). The round leaf mutant is an excellent marker although positive identification can only be made by observations of the first trifoliolate leaf. The primary leaves sometimes express the rounded apexes. Expression of the rounded apex always occurs in the lateral leaflets, while the terminal leaflet may express the character, but usually does not. The rounded apex of the lateral leaflet may be seen as soon as the individual leaflets are distinguished in the expanding leaves.

Figure 3-4 Round leaf mutant (rnd). A. Note the rounded apex of both primary leaves. B. Trifoliolate leaf from rnd at first anthesis. Note the rounded apex of all leaflets.


A


B

Dwarf Outcrossing

The mutant phenotype includes precocious trifoliolate leaf development and expansion, as well as reduced area of leaf lamina. A dwarf outcrossing plant (do) develops two fully expanded trifoliolate leaves in the same time period required for 7-1404 plants to develop one trifoliolate leaf to a very early stage of expansion (Figure 3-5A). At flowering, do plants average 140 leaves per plant, about 5 times more than 7-1404, and have twice the total leaf area (Table 3-3). The do plants have shorter internodes and are more highly branched than 7-1404 (Figure 3-5B). Dwarf outcrossing pods are shorter and the seeds are smaller than 7-1404. Progeny tests of do have revealed that the frequency of natural outcrossing ranges from 10 to 56% under field conditions. The mechanism of outcrossing is being determined.

The potential benefits from exploiting the high outcrossing trait in P. vulgaris for population improvement provide an exciting prospect. Zaumeyer (1972) expressed concern that snap bean cultivars are mainly derived from 3 sources, and are therefore highly susceptible to disease epidemics. A mechanism that facilitates hybridization would greatly help in increasing the genetic variability of beans.

Chlorotic Cup Leaf

The characteristics of this mutation are expressed when the leaf blade becomes concave and forms a shallow "watch glass" structure. The first indication of this mutant is the unveiling of the primary

Figure 3-5. Dwarf outcrossing mutant (*do*). A. Seedlings of 7-1404 (left) and *do* (right) 2 weeks from sowing (the primary leaves were removed). The *do* mutant has a distinct precocious leaf development over 7-1404.
B. Comparison of 7-1404 (left) and *do* (right) showing the shorter internodes and highly branched stem structure of *do*.

B


A

leaf blade and its discoloration (Figure 3-6A). Trifoliolate leaves emerge with no mutant characteristics (Figure 3-6B); only when a leaflet is fully expanded does cupping occur with chlorophyll deficiency patterns similar to those produced by manganese deficiency (Figure 3-6C and 3-6D).

Diamond Leaf

The name of this mutant is indicative of its very angular leaf shape (Figure 3-7B). The leaflets lack the broad rounding of the margin found in 7-1404 and their primary leaves have a distinct triangular shape (Figure 3-7A). Terminal leaflets resemble a diamond in shape (dia) and the lateral leaflets have angular sides, the shape being skewed from that of a diamond.

When making crosses with dia, poor pod set resulted when dia was the seed parent. Observation of dia flower structures revealed normal morphology. Adequate seed set from self-pollinated flowers was observed. A possible explanation is that the style of the dia mutant is more brittle than that of 7-1404 and is easily damaged during emasculation. Small hairline cracks were observed in the styles of several emasculated dia flowers.

Yellow Green

This mutant is classified as a chlorophyll mutant because its foliage is uniformly pale yellow. The phenotype is clear after primary leaves are fully expanded. Inheritance may be conditioned by

Figure 3-6. Chlorotic cup leaf mutant (cc). A Ten day old seedling of cc showing the characteristic epinastic cupping of the primary leaves. B Newly emerged cc trifoliolate leaf before the development of noticeable characteristics. C Trifoliolate leaf half way to full character expression, showing characteristics of leaf cupping and savoying. D Leaf with full expression of the cc characteristic with signs of interveinal chlorosis.

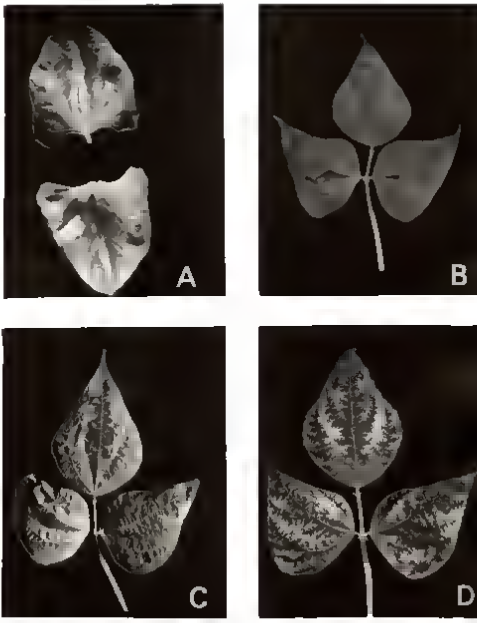

A
B
C
D

Figure 3-7. Diamond leaf mutant (dia). A. Seedling of dia 10 days from sowing showing the characteristic triangular shape of the primary leaves. B. Trifoliolate leaf showing the very angular leaf shape and the diamond shaped terminal leaflet.


A


B

duplicate recessive genes ($yg_1$ and $yg_2$) giving a $F_2$ segregation ratio of 15 normal to 1 mutant. The possibility of differential gametophyte survival has not been ruled out as a possible explanation for the transmission rate of this mutant. Further tests are in progress.

Progressive chlorosis

Expression of the character begins on leaves that are nearing full expansion. Emerging and expanding leaves are of a normal green color. There is a color gradient for yellowness in progressive chlorosis (pc) plants: normal color at the apex of the plant where leaves are emerging and beginning to expand, pale yellow color of nearly fully expanded leaves, and yellow fully expanded leaves. The color of the leaves is also be correlated with age, the older leaves being more yellow. Primary and secondary veins are green, giving the appearance of interveinal chlorosis.

Silver Leaf

This mutant is expressed in the seedling stage. When the primary leaves are free from the seed, they are milky yellow in color, which becomes silvery upon expansion. Under the light intensity of field conditions, the silver leaf mutant (sil) is more compact than T-1404. Thus, sil plants cannot compete well against normal foliage plants in mixed stands. The sil mutant grows normally under glass, indicating that UV light probably plays an important role in the stunted growth observed in the field. The inheritance ratio of the silver leaf mutant deviated from a 3:1 ratio. From several crosses with other

mutants made over 2 years, ratios were calculated (Table 3-41). A normal to mutant ratio of 4.48 ± 0.01 to 1.0 was observed for 1982, while for 1983 a ratio of 5.78 ± 0.50 to 1.0 was observed. Crosses of oil with 7-1404 resulted in similar ratios over the 2 year period with values of 5.1:1 and 5.2:1 for 1982 and 1983, respectively. Gametophytic effects influenced by environmental factors are suspected to produce these aberrant ratios.

Chlorotic Stem

Chlorotic stem (cs) is unique in that phenotypic expression remains visible well into plant maturity. The hypocotyle of newly emerged seedlings are pink near the base and white at the cotyledons and epicotyl. The stems, petioles and pods of older plants are creamy white. The leaves of cs are slightly chlorotic and classified as viridis. Primary leaves do not orient toward the sun even in strong sunlight.

Table 3-4 Phenotypic $F_2$ segregation ratios of the silver leaf mutant for several crosses over 2 years.

| Year | Cross | Phenotypic segregation: Normal | Silver leaf | Total | % Silver leaf plants | Ratio: Normal : Silver leaf |
|---|---|---|---|---|---|---|
| 1982 | sw x sil | 431 | 125 | 556 | 22.5 | 4.4 : 1 |
| 1982 | sl x sil | 328 | 92 | 420 | 21.9 | 4.6 : 1 |
| 1982 | sil x rnd | 401 | 114 | 515 | 22.1 | 4.5 : 1 |
| 1982 | gl x sil | 410 | 120 | 530 | 22.6 | 4.4 : 1 |
| Mean | | | | | 22.3±0.33 | 4.48±0.10 : 1 |
| 1983 | cc x sil | 534 | 94 | 628 | 15.0 | 6.7 : 1 |
| 1983 | sil x sl | 427 | 84 | 511 | 16.4 | 6.1 : 1 |
| 1983 | pc x sil | 507 | 81 | 588 | 13.8 | 7.3 : 1 |
| 1983 | sil x die | 562 | 92 | 654 | 14.1 | 7.1 : 1 |
| Mean | | | | | 14.8±1.17 | 6.78±0.50 : 1 |
| 1982 | sil x 7-1404 | 319 | 78 | 397 | 19.6 | 5.1 : 1 |
| 1983 | sil x 7-1404 | 550 | 130 | 680 | 19.1 | 5.2 : 1 |
| Mean | | | | | 19.4±0.35 | 5.15±0.07 : 1 |

CHAPTER IV

# LINKAGE RELATIONSHIP OF 10 INDUCED MUTANTS OF COMMON BEAN, *Phaseolus vulgaris* L.

## Introduction

The most extensive linkage map of common bean was reported by Lamprecht (1961), who described 8 linkage groups involving 26 genes (Figure 4-1). Many of these genes control color of the testa and corolla. These phenotypes are difficult to use as markers because of the complex complementary and/or modifying gene effects involved in their inheritance.

No concerted effort has been made to extend the genetic map for common bean since Lamprecht's work. Of the 211 characters reported by Roberts (1982), only 26 have been assigned to any linkage group. With a scarcity of good genetic markers to define the major linkage groups, the problem of assigning a new gene to a particular linkage group is compounded.

This investigation establishes the linkage relationships among 10 induced mutants to extend the known linkage relationships in common bean. Linkage tests between the *y* and *de* loci and several induced mutants begin the incorporation of the new mutants with Lamprecht's linkage map.

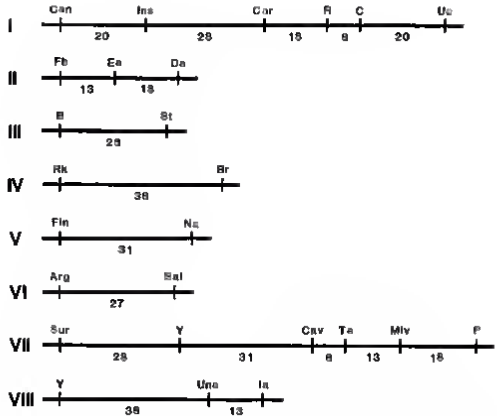



Figura 4-1. Lamprecht's proposed map of 8 linkage groups of Phaseolus vulgaris.

## Materials and Methods

Ten mutants, whose inheritance and phenotypes have been described in the previous chapter, were used for this study. The 10 mutants were intercrossed in a diallel set without selfs or reciprocal crosses, since inheritance tests of all 10 mutants indicated no cytoplasmic factors were involved in character expression.

Two hybrid seeds were planted in each of 8 pots for each combination in the diallel. Upon emergence and expansion of the primary leaves, or as soon thereafter as mutant expression began, any accidental selfs (showing the maternal mutant phenotype) were removed. The hybrid plants ("normal" type appearance) were thinned to 1 per pot as needed. Six hybrid plants were grown to maturity to produce $F_2$ seeds which were harvested in bulk. Six hundred seeds from each diallel combination were planted in the field on raised beds spaced 1.2 m apart. There were 2 rows per bed spaced 30 cm apart, and the plants were spaced 10 cm apart in the rows. Plants were scored when the 4 phenotypic classes could be distinguished, which varied from 3 to 6 weeks from planting.

The observed $F_2$ phenotypic ratios for each cross combination were tested against the expected ratio of 9:3:3:1 (for 2 unlinked recessive genes) using the chi-square goodness of fit test (Little and Hill, 1978). The precise value of P (probability of a greater value) was determined using a TI 59 calculator. Linkage values were calculated using the product ratio method of Fisher and Balmukand (1928), for crosses with significant chi-square values. The tables of Immer (1930) and Stevens (1933) were used to convert the product ratio to

percent recombination as a measure of linkage. When linkage was detected by repulsion phase testing, plants of the double mutant combination were recovered (if possible) and used for the coupling phase test. Three additional crosses were made to test for linkage: round leaf (rnd) x dwarf seed (dw), rnd x yellow wax pod (y), and dark green savoy leaf (dgs) x y.

The maximum likelihood method, as discussed by Allard (1956), was employed to combine data from 2 or more years as well as those of coupling and repulsion phase tests to provide a single linkage estimate. This method also determines the homogeneity of the individual sets of data. The chi-square and P value of Table 4-1 reflect this determination and from it we can evaluate whether the estimates are similar.

## Results and Discussion

Four linkage groups involving five mutants were observed among 45 $F_2$ repulsion diallel crosses (Table 4-1). Three mutants were linked into one group, which was then incorporated into a larger established linkage group (Figures 4-2 and 4-3). Two other mutants formed another linkage group (Figure 4-2).

Crosses of dgs with rnd revealed linkages of 25.3 and 24.9% recombination. The 31.6% recombination for the coupling test (Table 4-2) is in agreement with that of the repulsion tests. A recombinational value of 30.07% was calculated for data summed over tests and years (Table 4-3).

Table 4-1. Linkage relationships among several mutants determined from $F_2$ segregation data in repulsion phase tests.

| Cross mutant 1 | X | mutant 2 | Year | Phenotypic classification: Normal | Mutant-1 | Mutant-2 | Double mutant | $\chi^2$ df = 3[a] | P | Product ratio | Map units[y] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| dgs | X | rnd | 1981 | 278 | 110 | 126 | 8 | 31.040 | <0.001 | 0.1490 | 25.29 ± 2.72 |
| dgs | X | rnd | 1983 | 523 | 217 | 204 | 19 | 39.220 | <0.001 | 0.2245 | 29.96 ± 1.96 |
| rnd | X | el | 1982 | 245 | 131 | 118 | 1 | 54.703 | <0.001 | 0.0161 | 8.88 ± 3.01 |
| dgs | X | el | 1982 | 271 | 148 | 130 | 9 | 53.055 | <0.001 | 0.1226 | 23.12 ± 2.87 |
| pc | X | die | 1982 | 298 | 154 | 110 | 1 | 58.049 | <0.001 | 0.0176 | 9.28 ± 2.81 |
| pc | X | die | 1983 | 439 | 234 | 220 | 0 | 100.376 | <0.001 | | 1.10 ± 3.24[x] |

[a] Chi-square test for 9 normal:3 mutant-1:3 mutant-2:1 double mutant segregation ratio in the $F_2$.
[y] Units expressed as percent recombination.
[x] Map units estimated by maximum likelihood equation.

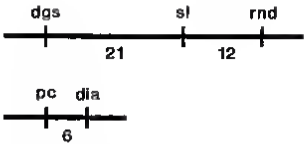



Figure 4-2. New linkage relationships of induced mutants of *Phaseolus vulgaris*

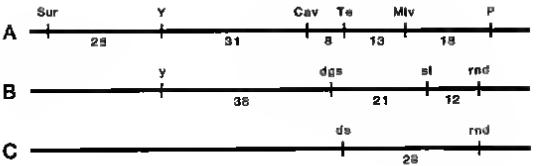



Figura 4-3. Known and proposed genes of linkage group VII of common bean.

Table 4-2. Linkage relationships among several mutants determined from $F_2$ segregation data in coupling phase tests.

| Mutant 1 + | Mutant 2 | X 7-1404 | Year | Phenotypic classification: Normal | Mutant 1 | Mutant 2 | Double mutant | $\chi^2$ 6f = 3[z] | P | Product ratio | Map units[y] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (dgs + rnd) | | X | 7-1404 | 1983 | 327 | 66 | 80 | 67 | 94 50 | <0 001 | 0 2246 | 31.56 ± 1.20 |
| (5gs + al) | | X | 7-1404 | 1983 | 315 | 47 | 43 | 80 | 273.26 | <0.001 | 0.0764 | 20.47 ± 0 98 |
| (rnd + al) | | X | 7-1404 | 1983 | 419 | 22 | 41 | 138 | 622 03 | <0 001 | 0 0216 | 11 56 ± 0 67 |

[z] Chi-square test for 9 normal:3 mutant-1:3 mutant-2:1 double mutant segregation ratio in the $F_2$

[y] Units expressed as percent recombination.

Table 4-3. Determination of recombinational values from coupling and repulsion phase data over several years employing the maximum likelihood equation

| Mutant characters in linkage | Recombinational value (%) | $\chi^2$ test[a] | df | P |
|---|---|---|---|---|
| dgs with rnd | 30 07 ± 1 38 | 2 027 | 2 | 0.36 |
| dgs with sl | 20.50 ± 1 34 | 1.217 | 1 | 0.27 |
| rnd with sl | 11 51 ± 0 35 | 0.614 | 1 | 0.43 |
| pc with dis | 6 35 ± 2.55 | 1 301 | 1 | 0 25 |

[a] Test of homogeneity among the data sets used to determine the recombinational value

The repulsion phase cross of rnd with al resulted in 9.3% recombination (Table 4-1), and 11.6% recombination was obtained for the coupling phase test (Table 4-2). Combining coupling and repulsion data sets resulted in a linkage estimation of 11.5% recombination, with homogeneity at P = 0.83 (Table 4-3).

The repulsion test of gna with el resulted in 23.1% recombination (Table 4-1), and the coupling test resulted in 20.5% recombination (Table 4-2). Combining the data sets resulted in a value of 20.50, with homogeneity at P = 0.27 (Table 4-3).

Linkage values for pc and dic were 9.3 for 1982 and 0 for 1983 (Table 4-1). No linkage could be estimated for 1983 by the product method because all four phenotypic classes must be represented to make the calculation. If a class is not represented in the $F_2$ population, then the linkage estimate becomes zero. The maximum likelihood method was used to determine linkage, since a zero value can be accommodated. A linkage value of 1.1 ± 3.24% was then obtained. A recombinational value of 6.35% resulted when both data sets were combined (Table 4-3). Additional tests are needed to improve the estimated value of this close linkage, which is probably overestimated at 6.35%.

Rnd was used as a marker to eliminate self pollinated seeds in the study of the dwarf seed character. The crosses of dwarf seed x round leaf revealed a linkage of 34.3% from $F_2$ repulsion data (Table 4-4). Coupling data involving 2666 $F_2$ plants revealed a linkage value of 29.3% recombination (Table 4-4). Combining the repulsion and coupling data sets gave an estimated linkage value of 30.39 (Table 4-4). The test for homogeneity indicated an inconsistency between the data sets. Due to the size of the coupling test and the better

Table 4-4 Determination of recombinational values from repulsion and coupling phase and from the maximum likelihood equation, involving the characters, dwarf seed (ds) and yellow wax pod (y)

| Characters in linkage | Year | Phenotypic classification: Normal | Mutant-1 | Mutant-2 | Double mutant | $\chi^2$ df = 3[x] | P | Product ratio | Map units[y] |
|---|---|---|---|---|---|---|---|---|---|
| **Repulsion** | | | | | | | | | |
| ds X rnd | 1981 | 339 | 124 | 111 | 13 | 17 302 | <0.001 | 0.3202 | 34 30 ± 2 42 |
| dga X y | 1983 | 346 | 141 | 136 | 13 | 13 396 | <0 005 | 0 4805 | 37 74 ± 2 24 |
| rnd X y | 1983 | 439 | 187 | 146 | 44 | 9 623 | <0.022 | | |
| **Coupling** | | | | | | | | | |
| ds X rnd | 1983 | 1733 | 331 | 304 | 294 | 264.39 | <0.001 | 0.2001 | 29.29 ± 0.91 |

**Maximum likelihood**

| Mutant characters in linkage | Recombinational value (%) | Chi-square test[x] | df | P |
|---|---|---|---|---|
| ds X rnd | 30.39 ± 1.06 | 15 362 | 1 | 0.001 |

[a] Chi-square tested for 9 normal:3 mutant-1:3 mutant-2:1 double mutant segregation ratio in the $F_2$.
[y] Units expressed as percent recombination.
[x] Test of homogeneity among the data sets used to determine the recombinational value.

estimation it provides (immer, 1930), the coupling test value was accepted as being the closer estimate of linkage.

The linkage value for dgs with y was 37.7% recombination (Table 4-4). This linkage estimate has not been confirmed by a coupling test. The linkage test for rnd with y indicated that these genes are independent (Table 4-4). If rnd were located to the left of dgs, a tight linkage between rnd and y would be expected (Figure 3-3b)

From the linkage of dgs with y it can be determined that dgs, ml and rnd are located in Lamprecht's linkage group VII (Figure 4-3A) Figure 4-3b is a map of the y, dgs, ml, rnd linkage group. The linkage group dgs - ml - rnd is proposed to lie between y and F, i.e., it lies to the right of y. We base our argument for this orientation on the assumption that te and ds are identical (bennett, 1982) and the linkage between ds and rnd. If the dgs - ml - rnd linkage group were to the left of y, then rnd would be independent of ds. It can be speculated that dgs is very close to te and cav; ml is close to miv; and rnd is very close to the basic color gene F; provided all the distances are consistent. It is clear that dgs, ml and rnd can be combined with cav, te, miv and F to form a single linkage group.

The linkage distance in Figure 4-3C between ds and rnd was determined by a coupling phase test. When the common locus rnd is aligned in Figures 4-3B and 4-3C the position of ds on Lamprecht's map can be estimated. If ds is located to the left of rnd, ds aligns with te of Lamprechts' map. This relationship provides interesting

possibilities for discussion. Both Freytag (1979) and Bassett (1982) reported on the confusion and uncertainty of the source of *de*. Bassett (1982) has speculated that *te* as reported by Lamprecht (1952) and the "eunis" effect reported by Tschermak (1931) are probably both controlled by the same gene, herein designated *ds*. A direct allelism test of *de* and *ts* is not possible since a properly marked seed stock of *te* is no longer extant (Blixt, personal communication). An allelism test can be approximated by comparison of map distances between *de* and various loci linked to *te*. Thus, if *de* has linkages to *P* and *y* that correspond, respectively, to the linkages of *te* to *P* and *y*, it is highly probable that *ds* is allelic with *te*.

CHAPTER V
OUTCROSSING IN COMMON BEAN ASSOCIATED WITH A
DWARF PLANT HABIT

## Introduction

Autogamy can create a narrow genetic base in crop plants, which increases the genetic vulnerability to pests and pathogens (Zaumeyer, 1972). If cross-pollination mechanisms were available in Phaseolus vulgaris, they could be used in breeding programs to break genetic linkages and increase recombination (Rutger and Beckham, 1970; Ibrahim and Coyne, 1975; Webster et al., 1977; Mansbardt, 1980).

Natural outcrossing in common bean is usually reported to range from 0 - 10% depending on location, environment, and availability of pollinating insects (Nackle and Smith, 1935; Barrons, 1939; Tucker and Harding, 1975). These natural hybrids ("rogues") are an important problem in the maintenance of purity of commercial seeds, but the sporadic occurrence and usual low frequency of these hybrids make them infeasible for use in a breeding project.

Crosses between Phaseolus vulgaris and P. coccineus have been made to incorporate the cross-pollinating mechanism of P. coccineus into P. vulgaris (Rutger and Beckham, 1970; Ibrahim and Coyne, 1975; Mansbardt, 1980). Limited success has been obtained from these studies.

The use of male sterility as a cross-pollination mechanism has also met with limited success in common bean (Agba and Wood, 1977;

Bannerot, 1980; Mutschler and Bliss, 1980; and MacKenzie, 1984)
Genetic male sterility is usually controlled by a recessive gene, and considerable time is needed to locate male sterile plants in segregating populations. The classification must be done during the 1 to 2 week bloom period, which severely limits the size of an outcrossing population from which seed will be harvested only from male-sterile plants.

Wyatt (1982) reported no indehiscent anther character of beans controlled by a recessive gene. Ninety-four percent of 553 progeny from anther indehiscent plants were hybrids. A major problem with this character is its unstable expression in heterozygous plants, i.e., some do, some do not (Wyatt, unpublished data).

Cytoplasmic male sterility has been found along with numerous good maintainer genotypes, but only one "good" restorer genotype has been identified (MacKenzie, 1984). Fertility restoration is controlled by 3 genes in a complex complementary mode. Pollen fertility is not completely restored in the $F_1$ progeny of cyto-ms X restorer. The search continues for a more simply inherited source of restoration.

A major problem of genic male sterility or indehiscent anthers is the great labor required for identification of sterile or indehiscent plants in a segregating population. Such identification is necessary if seed is to be harvested only off the sterile plants, i.e., only outcrossed seed (heterozygous for sterility) is to be advanced to the next generation. In the present work an induced mutant of Phaseolus vulgaris which yields 10 to 50% sterility

outcrossed seed was evaluated for the mechanism(s) responsible for the outcrossing behavior. This outcrossing behavior is associated with a dwarf plant habit, which serves as a strong marker character identifying those plants with outcrossing behavior at all stages of development including full senescence.

## Materials and Methods

Dwarf outcrossing (*dc*), a 10 kR gamma-ray induced mutant of Florida dry bean breeding line, 7-1404, was observed to have high numbers of spontaneous hybrid seeds from field grown material. Since *dc* is a recessive marker, we made use of the fact that any cross-pollination would result in the *dc* locus becoming heterozygous and the $F_1$ progeny expressing the dominant normal phenotype. Hence, the progeny from such *dc* plant will segregate for two phenotypic classes: the *dc* phenotype (*dc*/*dc*) resulting from self pollination and the normal phenotype (+/*dc*) resulting from cross-pollination.

Seeds from field grown *dc* plants were harvested from 3 different plantings grown in 3 different years (1980 - 1982) to determine the range of outcrossing under field conditions. Seeds were harvested by bulk or single plants, and were planted in Speedling trays. Fourteen days after sowing, seedlings were scored for either self-pollination, characterized by the *dc* phenotype, or cross-pollination, characterized by the normal phenotype (Figure 3-5A).

Several mechanisms for outcrossing are known in the genus *Phaseolus*. Experiments were conducted to determine the mechanism(s) involved in outcrossing of *dc*.

The first experiment conducted was to determine pollen viability. This was accomplished by staining the pollen using potassium iodide (IK) or acetocarmine. Three to four mature buds (anthesis would occur in 1 to 2 days) from each of 10 plants were tested. Pollen grains that stain dark brown with acetocarmine or black with IK were considered viable (capable of fertilizing an ovule). Results of this test would indicate whether pollen abortion is responsible for outcrossing.

Pollen shed below the stigma on the stylar brush is responsible for the failure of self-pollination in P. coccineus. When pollinating insects trip the flowers, they may foreign pollen with "self" pollen, resulting in partial outcrossing. We protected 15 dc plants, each in screened greenhouses or field cages, from pollinating insects. As a control 15 dc plants were grown, unprotected in the field. Observations were made on pod set and time required for pod set.

From the protected dc plants, 1000 seeds were planted to determine if instability of the dc character contributed to the observed numbers of normal phenotypes. The placement of pollen and stigma type are an important consideration in the outcrossing of P. coccineus. Several flowers from each of 10 greenhouse and 30 field grown plants were examined for pollen placement and stigma type with a 30X hand lens. No quantitative measurements were made because of the difficulties involved.

Preliminary observations of the absence of pollen on the stigmatic surface (or complete indehiscence) of some dc flowers in the early morning hours, led to the hypothesis of delayed anther

dehiscence as the cause for outcrossing under field conditions. This led to a series of experiments described below in which unemasculated, open flowers of dw were manually crossed with 'Sprite' or 7-1404 pollen at hourly intervals for several hours to test the hypothesis of delayed anther dehiscence. If delay of anther dehiscence was the cause for outcrossing, the percentage of outcrossing should decline with increasing time from flower opening.

Prior to anthesis, all dw plants were protected in insect proof greenhouses or portable screen cages to prevent insect pollination. All pollinations were done in the following manner. Open flowers from dw plants were manually pollinated without emasculation with pollen of 'Sprite' or 7 1404 and labeled with the time and date of pollination. The potential for outcrossing was determined by making successive manual pollinations for 20 minutes at hourly intervals unless otherwise specified. Pollinations continued until new pods failed to set, i.e., the plant had a full pod set.

The $F_1$ seeds from the above manual pollination were handled and evaluated in the following manner. Mature dry seeds were harvested and planted in Speedling trays by individual pods, time of pollination and plant number. The percentage of cross-pollination was scored 14 days after planting as previously mentioned.

Greenhouse Experiment 1

Seven dw plants on 7-1404 background were grown in 15 cm pots in vegetable plug mix medium. Standard cultural practices were followed according to Florida conditions. Seeds were planted December 29,

1981, and anthesis began on February 5, 1982. All open flowers were pollinated with 'Sprite' pollen between 0800 HR and 0900 HR.

Field Experiments

Two plantings of do on 7-1404 background were made at the Horticultural Unit with 25 plants each. The first planting (Field Experiment 1) was made in early April and the second (Field Experiment 2) in late July. Pollinations were made at 5 hourly intervals from 0630 HR to 1030 HR for the first planting and between 0730 HR to 1430 HR for the second planting. Percent of flowers with and without anther dehiscence were observed for the first planting.

Greenhouse Experiment 2

Ten do plants on 7-1404 background were grown as previously described for the greenhouse. Anthesis began in early January and open flowers were pollinated starting at 0730 HR and ending at 1230 HR. Percent of flowers with and without anther dehiscence were recorded.

## Results and Discussion

Outcrossing Rates for Field Grown do Plants

The 7 original do mutants discovered in the $M_2$ generation (field, 1980) gave cross-pollination frequencies of 5 to 45% for $M_3$ progeny tests (Table 5-1). The average cross-pollination of the 7 plants was 23% (Table 5-2). All 7 plants came from a single $M_1$ parent selection and are considered identical in genotype since they arose from a

Table 5-1. Percent outcrossing in progenies from the 7 original dwarf outcrossing mutants from the $M_2$ generation grown in the field in 1980.

| Plant No. | Phenotypic classification | | Percent outcrossing |
|---|---|---|---|
| | Normal | Dwarf | |
| 1 | 20 | 23 | 47 |
| 2 | 6 | 37 | 14 |
| 3 | 2 | 42 | 5 |
| 4 | 20 | 22 | 48 |
| 5 | 6 | 42 | 13 |
| 6 | 7 | 39 | 15 |
| 7 | 4 | 17 | 23 |

Table 5-2. Percent of outcrossing observed in progenies derived from dwarf outcrossing plants grown in the field during 3 different years.

| Generation | Phenotypic classification | | Percent outcrossing |
|---|---|---|---|
| | Normal | Dwarf | |
| M2 (1980) | 65 | 222 | 23 |
| M5 (1981) | 225 | 165 | 56 |
| M7 (1982) | 24 | 219 | 10 |

single mutation event. Plant variation for outcrossing is attributed to variability of insect pollination activity.

A cross pollination rate of 56% was observed in progenies from field grown do plants in 1981 (Table 5-2). No range is given because seeds of several plants were harvested in a single bulk lot. A third field planting, grown in 1982, averaged 10% cross-pollination for 16 plants tested (Table 5-2). The variation in outcrossing is attributed to the year to year variation in the average population density of bumble bees in the field at the time of pollination. Environmental effects such as rain, would reduce the probability of outcrossing by limiting bee activity during the limited pollination period. The drought in 1981 - 1982 may also have reduced the population of bumble bees in 1982 by contributing to death of the bee larva in the soil.

Testing for Mechanisms That Lead to Outcrossing

Experiments to determine the mechanism involved in outcrossing of do have led to several observations. Pollen staining with IK or acetocarmine resulted in 90 - 96% pollen stainability, based on 3 to 4 buds from each of 10 plants. The results indicate a high pollen fertility (Bassett, unpublished data). This effectively eliminated pollen sterility as the cause of outcrossing of do plants.

No difference in the number of pods set or period of time necessary for full pod set was observed between plants grown in screened greenhouses or field cages when compared to unprotected

greenhouse or field grown plants. This implies that neither pollen sterility nor any other mechanism leading to a failure of self-pollination can explain the outcrossing observed. Thus self-incompatibility is eliminated as a possible factor.

About 1000 seeds produced on do from plants protected from insect pollinators were planted to determine whether unstable expression of the mutant was a factor for the observed numbers of normal phenotypes. No normal types were observed (Table 5-3), which indicates that neither phenotypic instability nor genetic reversion of the do character is responsible for the normal phenotypes in the progeny of field grown do plants.

The frequency of outcrossing observed in do plants was similar to that reported for P. coccineus, in which outcrossing estimates ranged from 30 to 70% (Hirsede and Evans, 1973; Smartt, 1976). Although the percentage of outcrossing was similar for the do mutant and P. coccineus, the mechanism was found to be quite different. Outcrossing of P. coccineus is related to the extrorse stigma position and pollen placement away from and below the stigmatic region (Hawkins and Evans, 1973). The do mutant has an introrse stigma position and pollen is shed on the stigmatic surface as in normal P. vulgaris (Webster et al., 1977). Since attempts to transfer the extrorse stigma type to P. vulgaris have met with limited success, the do mutant may provide the desired high rates of outcrossing with several advantages over the P. coccineus type flowers.

Delay of anther dehiscence. A surprising observation was the lack of observable pollen on a number of styles of newly opened do

Table 5-3. The percentage of normal segregants (or mutants) observed in progenies from dwarf outcrossing plants protected from pollinating insects by a screened greenhouse or cages in the field.

| Generation | Phenotypic classification | | Percent outcrossing |
|---|---|---|---|
| | Normal | Dwarf | |
| Greenhouse | | | |
| M4 (1980) | 0 | 324 | 0 |
| M5 (1981) | 0 | 295 | 0 |
| Field | | | |
| M7 (1982) | 0 | 364 | 0 |

flowers examined at early morning. According to Weinstein (1926), Free (1966), and Webster et al. (1977), pollen shed occurs before or at flower opening for P. vulgaris; thus it is highly self-pollinated due to cleistogamy. If anther dehiscence was delayed for some time after flower opening, the possibility for hybridization would be significantly increased.

Greenhouse experiment 1. An average of 33% cross pollination was obtained by crossing open, non-emasculated flowers of do plants with pollen of 'Sprite' between 0800 and 0900 HR in the greenhouse in February (Table 5-4). This indicates that significant hybridization can and does occur after anthesis if foreign pollen is transferred onto the stigmatic surface.

Field experiment 1. The percent of cross-pollination observed over a 5 hour period revealed cross-pollination of 86% at 0730 HR and 51% at 1030 HR when pollen of 7-1404 was manually transferred to open, non-emasculated do flowers (Table 5-5). The average value over the 5 hour period was 64%. All plants were protected from insect pollination by screen cages in the field.

Observations of open do flowers for anther dehiscence with a 30X hand lens between 0630 and 1030 HR revealed significant numbers of flowers without observable pollen (Table 5-6). At 0630 HR, 70% of the open flowers were found with no anther dehiscence. This level decreased to 28% at 1030 HR. Observation of 7-1404 at 0630 HR revealed all flowers with pollen dehisced from anthers.

Field experiment 2. In an effort to determine the extent of outcrossing of do, a second field planting was initiated. Significant

Table 5-4. Percentage of cross-pollination in $F_1$ progeny obtained by hand pollination of unemasculated open flowers of 7 dwarf outcrossing plants between 0800 and 0900 HR.

| Plant No. | Phenotypic classification | | Percent cross-pollination[z] |
|---|---|---|---|
| | Normal | Dwarf | |
| 1 | 19 | 21 | 48 |
| 2 | 15 | 55 | 21 |
| 3 | 26 | 25 | 51 |
| 4 | 2 | 53 | 4 |
| 5 | 36 | 46 | 44 |
| 6 | 12 | 20 | 38 |
| 7 | 20 | 50 | 29 |

[z]The manually applied pollen was from plants homozygous for wild type at the *do* locus.

Table 5 5.. Percentage of cross-pollination in $F_1$ progeny from dwarf outcrossing plants where open flowers were hand pollinated without emasculation between 0630 and 1030 HR. Field grown, April planting.

| Pollination time (HR) | Phenotypic classification | | Percent cross-pollination[a] |
|---|---|---|---|
| | Normal | Dwarf | |
| 0630 | 34 | 38 | 55 |
| 0730 | 38 | 6 | 86 |
| 0830 | 60 | 40 | 60 |
| 0930 | 71 | 30 | 72 |
| 1030 | 23 | 22 | 51 |
| Total | 226 | 126 | |
| Mean | | | 64 |

[a]The pollen manually applied was from plants homozygous for wild type at the dc locus

Table 5-6. Percentage of anther dehiscence from dwarf outcrossing plants observed between 0630 and 1030 HR. Field grown, April planting.

| Time (HR) | No. flowers scored | Percent of flowers | |
|---|---|---|---|
| | | Dehisced | Indehiscent |
| 0630 | 125 | 30 | 70 |
| 0730 | 123 | 50 | 50 |
| 0830 | 110 | 70 | 30 |
| 0930 | 119 | 66 | 34 |
| 1030 | 100 | 72 | 28 |

cross pollination was observed for all time periods from 0730 to 1430 HR (Table 5-7). The range of cross pollination was from 66% at 0730 HR to 26% at 1430 HR. Overall, the cross-pollination rate was 53% for the time period tested.

Comparison of the first and second planting indicates some similarities for corresponding time periods. Weinstein (1926) reported high temperature may be an important variable in the rapidity of fertilization once pollination had occurred. If this is true, with the higher temperatures of July we would expect a lower rate of cross-pollination due to rapidity of pollen tube growth after selfing when compared to April. The outcrossing rates for July were lower than April.

Greenhouse experiment 2. Manual pollination experiments done in the greenhouse in January between 0730 and 1330 HR resulted in a narrow range of cross-pollination rates (Table 5-8). Cross-pollination of 42% was the high at 0930 HR, with 27% at 1130 HR. The average outcrossing percentage was 35%. For the period of time tested, nearly all the open flowers had anthers that dehisced pollen (Table 5-9). Despite this fact, significant cross-pollination was observed. Weinstein (1926) reported that the time from pollination to fertilization was 8 to 9 hours. Free (1970) suggested that pollen tubes of foreign pollen grew more quickly than those of self-pollen; otherwise there would be no outcrossing. This theory would account for the high rate of observed cross-pollination, despite the high levels of dehisced anthers.

Table 5-7. Percentage of cross-pollination of $F_1$ progeny from dwarf outcrossing plants where open flowers were hand pollinated between 0730 and 1430 HR. Field grown, July planting.

| Pollination time (HR) | Phenotypic classification | | Percent cross-pollination[a] |
|---|---|---|---|
| | Normal | Dwarf | |
| 0730 | 39 | 20 | 66 |
| 0830 | 60 | 42 | 59 |
| 0930 | 46 | 40 | 54 |
| 1030 | 34 | 23 | 60 |
| 1130 | 59 | 44 | 58 |
| 1230 | 38 | 34 | 52 |
| 1330 | 47 | 53 | 47 |
| 1430 | 14 | 39 | 26 |
| Total | 337 | 295 | |
| Mean | | | 55 |

[a]The manually applied pollen was from plants homozygous for wild type at the *dw* locus

Table 5 8 Percentage of cross-pollination in $F_1$ progeny where open flowers of dwarf outcrossing plants were hand pollinated without emasculation between 0730 and 1230 HR. Green house grown, December planting

| Pollination time (HR) | Phenotypic classification | | Percent cross-pollination* |
|---|---|---|---|
| | Normal | Dwarf | |
| 0730 | 82 | 179 | 31 |
| 0830 | 77 | 158 | 33 |
| 0930 | 85 | 123 | 42 |
| 1030 | 74 | 132 | 36 |
| 1130 | 34 | 90 | 27 |
| 1230 | 90 | 136 | 40 |
| Total | 446 | 818 | |
| Mean | | | 35 |

*The pollen manually applied was from plants homozygous for wild type at the do locus.

Table 5-9. Percentage of anther dehiscence from dwarf outcrossing plants observed between 0700 and 1100 HR. Greenhouse grown, December planting.

| Time (HR) | No. flowers scored | Percent of flowers | |
|---|---|---|---|
| | | Dehisced | Indehiscent |
| 0700 | 100 | 93 | 7 |
| 0800 | 141 | 94 | 6 |
| 0900 | 144 | 93 | 7 |
| 1000 | 142 | 94 | 6 |
| 1100 | 129 | 99 | 1 |

Data from field pisetings and winter greenhouse plantings differed. Although durations of study differed, the field plantings had higher total cross-pollination percentages for any given time period and higher overall average than that of the greenhouse plantings (Tables 5-4, 5-5, 5-7, and 5-8). The reason for this difference is unknown at this time, but environmental factors such as temperature, which was discussed by Weinstein (1926), is suspected.

The outcrossing character was originally found in a mutant selected for dwarf plant habit. All 7 original $M_2$ plants possessed the outcrossing character (Table 5-2). Preliminary data of dc plant selections from $F_2$ segregating populations when manually pollinated with 7-1404 pollen indicate close linkage or pleiotropic association of the characters. Further tests are needed to establish whether the association of outcrossing with the dwarf plant habit is truly pleiotropic or closely linked.

The delay of anther dehiscence is not the same as the anther indehiscence character reported by Wyatt (1982). Flowers of anther indehiscent plants if protected from pollinators will not set pods as dc plants will. The anther indehiscence character is expressed by the absence of a suture from which the anther can normally dehisce. The dc character has anther dehiscence, but it is delayed by several hours when compared to normal plants. It is not known at this time if the heterozygous state of Dc expresses the outcrossing character as is sometimes the case with Wyatt's indehiscent anther under certain environmental conditions.

## Summary

Delay of anther dehiscense was found to be responsible for cross-pollination of up to 56% (highest average value observed in the field from insect pollination), 3 5 times higher than previously reported for field grown P. vulgaris The outcrossing character was found to be associated with a dwarf plant habit, which aids identification of outcrossing plants a matter of identifying the dwarf phenotype. In annual pollination studies, up to 86% cross-pollination was obtained, and significant cross-pollination was still occurring at mid-afternoon. This new mutant can be exploited in breeding programs to efficiently increase rates of genetic recombination. The outcrossing plants in a population segregating for do are conveniently "flagged" at all stages of plant development by the marker character, dwarf plant habit. The dwarf habit expresses as shorter internodes, shorter pods, more branching, smaller and more numerous leaves, and smaller seeds. When do is in a half-runner plant habit background, the do segregates are competitive with normal plants at typical plant population densities. The do segregates are not competitive with normal plants in a bush background (determinate habit) at typical plant densities.

## CHAPTER VI
## SUMMARY AND CONCLUSIONS

Gamma irradiation of dry seeds of common bean, *Phaseolus vulgaris* L., Florida breeding line 7-1404, resulted in the selection of 10 mutants suitable as genetic markers for linkage mapping. All the selected mutants have highly visible phenotypic characteristics. The 10 mutants included changes in leaf shape and texture, dwarfism and various chlorophyll deficiencies. Each of the selected mutant characteristics was controlled by a single recessive gene, except for yellow green which was controlled by 2 recessive genes. The round leaf (*rnd*), dark green savoy (*dgs*), diamond leaf (*dia*), chlorotic cup leaf (*cc*), and stipellues lanceolate leaf (*sl*) mutants are adequately described by their names. Dwarf outcrossing (*da*) has small leaves, short internodes and pods, and a natural outcrossing frequency of 10-50%. Chlorotic stem (*cs*) has milky white stems and pods, while silver leaf (*sil*) has its color modified by a silvery reflectance. Progressive chlorosis (*pc*) has leaves which emerge normal green in color, but become chlorotic with age. Yellow green ($yg_1$, $yg_2$) has a uniform yellow foliage color. Yellow green is believed to be controlled by 2 recessive genes, but gametophytic effects have not been ruled out at this time. The two recessive mutants were used for linkage testing in diallel crosses. Linkage was calculated using $F_2$ data, employing Fisher and Balmukand's product method for crosses in

repulsion and coupling phases (Fisher and Balmukand, 1982). The maximum likelihood equation was employed to combine repulsion and coupling data to estimate a single linkage value. Two linkage groups involving 5 mutant characters were discovered. Round leaf (rnd), stipelless lanceolate leaf (sl), and dark green savoy (dgs) formed one linkage group (dgs - 21 - sl - 12 - rnd), while dis and pc formed the second linkage group (dis - 5 - pc). Dgs was linked (38 map units) to the yellow wax locus, y, thereby tying the dgs - sl - rnd linkage group into linkage group VII defined by Lamprecht (1961). A coupling phase test has confirmed the linkage of rnd with dwarf seed (ds) at 29% recombination. Preliminary data suggest that the ds character may be the same gene as Lamprecht's tenuis (te), also of linkage group VII, if this is true, then the orientation of the dgs - sl - rnd linkage group is determined with respect to y and P, since rnd is independent of the y locus. The proposed linkage relationships of linkage group VII is speculative at this time, and hinges on the fact that te and ds are found at the same locus. More tests are being conducted, with the results becoming available in early summer. In one $M_2$ progeny 7 plants were discovered with a dwarf mutant phenotype (de). Tests of $M_3$ progenies revealed outcrossing rates of 5 to 47%. Pollen staining tests of the dwarf mutant indicated an unusual pollen abortion rate. Pod set from plants grown in insect-proof enclosures were comparable to unprotected ds plants. Data reveal that male sterility, self-incompatibility, pollen placement, or the need for tripping was not responsible for outcrossing in ds plants. The hypothesis that delay of anther dehiscence is responsible for the

elevated levels of cross pollination was presented. Open flowers of dwarf plants manually crossed between 0630 HR and 1430 HR with either 'Sprite' or 7-1404 pollen resulted in cross-pollination of 26 to 86%. The frequency of cross-pollination declined with time of day, supporting the hypothesis that delay of anther dehiscence is responsible for levels of outcrossing observed. Differences in levels of outcrossing were observed, with cooler periods of the year (spring and winter) having a higher level of outcrossing than warmer periods of the year (summer).

APPENDIX A

MUTANT CHARACTERS TESTED FOR MODE OF INHERITANCE BUT NOT SELECTED FOR DIALLEL TESTING OF LINKAGE DUE TO DIFFICULTIES IN DETERMINING GENETIC INHERITANCE AND CLASSIFICATION OF THE MUTANT IN SEGREGATING POPULATIONS

| Mutant | Year tested | Observed ratio | | $\chi^2$ (3:1) | P |
|---|---|---|---|---|---|
| | | Normal | Mutant | | |
| rumpled leaf | 1981 | 462 | 10 | | <0.001 |
| viridis AO-4 | 1981 | 452 | 109 | 9.2840 | 0.0023 |
| viridis YC-4 | 1981 | 433 | 138 | 0.2107 | 0.6462 |
| chlorotica-2 | 1981 | 401 | 114 | 2.2531 | 0.1333 |
| chlorotica-2 | 1982 | 249 | 91 | 0.5640 | 0.4526 |
| silver leaf | 1981 | 440 | 127 | 2.6577 | 0.1031 |
| dwarf cup leaf | 1982 | 312 | 113 | 0.4288 | 0.5126 |
| small leaf dwarf | 1982 | 333 | 66 | 13.8329 | <0.001 |
| chlorotica-1 | 1982 | 295 | 91 | 0.4580 | 0.4986 |
| yellow crown | 1982 | 296 | 65 | 9.7420 | 0.0018 |
| viridis AO-3 | 1982 | 308 | 94 | 0.5605 | 0.4541 |
| diamond type | 1982 | 324 | 106 | 0.0279 | 0.8673 |
| viridis YC-1 | 1981 | * | | | |
| bronze | 1982 | * | | | |
| diamond-1 | 1982 | * | | | |
| green leaf dwarf | 1982 | * | | | |
| semi-dwarf | 1982 | * | | | |
| dwarf leaf | 1982 | * | | | |
| glossy-3 | 1982 | * | | | |
| lanceolate-3 | 1982 | * | | | |
| dwarf chlorotica | 1982 | * | | | |

*Genetic ratio not given because classes could not be separated

APPENDIX B

MUTANT X MUTANT CROSSES (REPULSION PHASE TESTS) THAT REVEALED INDEPENDENT SEGREGATION FOR THE TWO CHARACTERS IN $F_2$ POPULATIONS.

| Cross | Year | Observed $F_2$ segregation ratio: Normal | Mutant 1 | Mutant 2 | Double mutant | $x^2$ (9:3:3:1) | P |
|---|---|---|---|---|---|---|---|
| 8gs X dia | 1981 | 303 | 115 | 106 | 38 | 1.6630 | 0.6452 |
| do X 8gs | 1981 | 316 | 120 | 110 | 30 | 2.5678 | 0.4632 |
| 8gs X ss | 1982 | 314 | 101 | 87 | 39 | 3.1613 | 0.3674 |
| dgs X pc | 1982 | 324 | 130 | 87 | 36 | 2.6860 | 0.4426 |
| dgs X cc | 1982 | 311 | 112 | 99 | 34 | 0.8665 | 0.8335 |
| dgs X sil | 1982 | 298 | 112 | 86 | 34 | 3.4271 | 0.3303 |
| rnd X dia | 1981 | 334 | 107 | 95 | 32 | 2.2473 | 0.5227 |
| do X dia | 1983 | 367 | 126 | 125 | 30 | 3.0069 | 0.3906 |
| dia X cc | 1982 | 332 | 118 | 88 | 37 | 4.8616 | 0.1822 |
| sil X dia | 1983 | 446 | 75 | 116 | 17 | 9.5469 | 0.0226 |
| rnd X do | 1981 | 317 | 104 | 122 | 33 | 2.3664 | 0.4999 |
| cs X rnd | 1982 | 271 | 78 | 90 | 27 | 1.5403 | 0.6730 |
| rnd X pc | 1982 | 207 | 76 | 78 | 19 | 2.3228 | 0.5062 |
| rnd X ss | 1982 | 304 | 103 | 113 | 46 | 4.3771 | 0.2235 |
| sil X rn8 | 1982 | 294 | 87 | 107 | 27 | 2.9752 | 0.3955 |
| cs X dn | 1982 | 335 | 100 | 107 | 29 | 2.3131 | 0.5100 |
| do X pc | 1982 | 312 | 101 | 91 | 41 | 2.7840 | 0.4261 |
| cs X do | 1982 | 250 | 87 | 68 | 21 | 4.7580 | 0.1904 |
| cc X do | 1983 | 350 | 115 | 115 | 48 | 2.1090 | 0.5501 |
| do X sl | 1982 | 225 | 77 | 85 | 21 | 1.8301 | 0.6084 |
| do X sil | 1982 | 298 | 112 | 86 | 34 | 3.4271 | 0.3303 |
| cs X pc | 1981 | 315 | 106 | 108 | 31 | 0.5523 | 0.9073 |
| sl X ss | 1982 | 329 | 102 | 96 | 30 | 2.1893 | 0.5341 |
| cs X sil | 1982 | 329 | 95 | 120 | 30 | 2.3629 | 0.5006 |
| pc X cc | 1982 | 350 | 114 | 121 | 27 | 3.7502 | 0.2897 |
| pc X sl | 1983 | 358 | 123 | 13 | 37 | 0.6013 | 0.8961 |
| ss X sl | 1983 | 363 | 122 | 117 | 39 | 0.1556 | 0.9844 |

APPENDIX B--CONTINUED

| Cross | Year | Observed $F_2$ segregation ratio | | | | $Chi^2$ | P |
|---|---|---|---|---|---|---|---|
| | | Normal | Mutant 1 | Mutant 2 | Double mutant | | |
| $ys_1$, $ys_2$ X dia[2] | 1983 | 337 | 27 | 94 | 6 | 5.7059 | 0.1268 |
| $ys_1$, $ys_2$ X rnd | 1982 | 423 | 21 | 142 | 3 | 6.0560 | 0.1084 |
| $ys_1$, $ys_2$ X rnd | 1983 | 805 | 42 | 220 | 7 | 4.5396 | 0.2086 |
| $ys_1$, $ys_2$ X pc | 1981 | 398 | 32 | 142 | 8 | 1.4429 | 0.6962 |
| $ys_1$, $ys_2$ X de | 1982 | 289 | 97 | 20 | 9 | 0.9071 | 0.8237 |
| $ys_1$, $ys_2$ X ce | 1983 | 445 | 135 | 33 | 8 | 1.7432 | 0.6274 |

[2]Chi square test ratio of 45 normal:3 mutant-1:15 mutant-2:1 double mutant for all crosses involving $ys_1$, $ys_2$

APPENDIX C

MUTANT X MUTANT CROSSES (REPULSION PHASE TEST) THAT INDICATE SIGNIFICANT DEVIATIONS FROM EXPECTED RATIOS, BUT GIVE NONSENSE VALUES FOR LINKAGE ESTIMATES INDICATING DISTURBANCES IN THE OBSERVED RATIOS.

| Cross | Year | Observed $F_2$ segregation ratio | | | | $\chi^2$ | |
|---|---|---|---|---|---|---|---|
| | | Normal | Mutant 1 | Mutant 2 | Double mutant | (9:3:3:1) | P |
| cs X die | 1982 | 356 | 84 | 87 | 26 | 15.0370 | 0.0018 |
| cs X die | 1983 | 428 | 109 | 99 | 27 | 20.0370 | <0.001 |
| dia X sl | 1982 | 321 | 83 | 114 | 27 | 7.1131 | 0.0684 |
| dia X sl | 1983 | 367 | 117 | 93 | 31 | 10.1656 | 0.0172 |
| cs X cc | 1982 | 289 | 95 | 82 | 18 | 7.0034 | 0.0718 |
| cs X cc | 1983 | 394 | 105 | 122 | 30 | 7.3048 | 0.0628 |
| pc X sil | 1983 | 411 | 96 | 74 | 4 | 60.5261 | <0.001 |
| cc X sil | 1983 | 401 | 133 | 92 | 2 | 49.4126 | <0.001 |
| sl X sil | 1982 | 255 | 73 | 61 | 31 | 6.7683 | 0.0797 |
| sl X sil | 1983 | 342 | 85 | 62 | 22 | 26.6026 | <0.001 |
| rnd X y | 1983 | 439 | 187 | 146 | 44 | 9.5232 | 0.0221 |
| $ys_1,ys_2$ X dgs[E] | 1982 | 365 | 11 | 136 | 3 | 12.8054 | 0.0051 |
| $ys_1,ys_2$ X cc | 1982 | 367 | 118 | 11 | 4 | 10.8067 | 0.0128 |
| $ys_1,ys_2$ X sil | 1983 | 688 | 57 | 112 | 5 | 127.5505 | <0.001 |
| $ys_1,ys_2$ X sl | 1982 | 305 | 102 | 8 | 5 | 7.7570 | 0.0513 |

[E] Chi-square test ratio of 45 normal 3 mutant-1 15 mutant-2 1 double mutant for all crosses involving $ys_1$, $ys_2$.

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He is married to the former Ms. Lynn M. Tsuteuma.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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